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Symbiotic Nitrogen Fixation

Prospects for Enhanced Application in Tropical Agriculture

Editor
Rachid Serraj

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Welcome Address

William D. Dar

Director General, ICRISAT

Distinguished guests, our partners and friends, ladies and gentlemen, dear colleagues. Good morning. It is my great pleasure to represent the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and deliver the opening address at this important workshop on the Biological Nitrogen Fixation Challenge Program initiative.

This workshop has one major objective – to define research strategies for a Challenge Program (CP) on biological nitrogen fixation (BNF) based on the concept note submitted to CGIAR's interim Science Council. The CP initiatives have been launched by the CGIAR to face growing global challenges facing world agriculture and food security. As recently defined, a Challenge Program is a *time-bound, independently governed program of high impact research that targets the CGIAR goals in relation to the complex issues of overwhelming global significance – or regional significance with global impact – and requires partnerships among a wide range of institutions to be successful*. All these criteria apply perfectly to the BNF Challenge Program initiative.

I will not stress too much on the current global challenges of agriculture, status of soil fertility and potential benefits of BNF, as I am sure you are all well aware of these facts. But as we are celebrating this same week the 5th Anniversary of the World Food Summit, let us remember that five years down the line, the situation of global food security has unfortunately not changed much. More than 800 million people are still poor and malnourished and live on less than a dollar a day. Two decades from now there may still be 600 million poor people who struggle to merely survive. The Green Revolution succeeded in doubling food production but did not solve the problems of malnutrition and hunger or wipe out poverty. It did not create enough jobs to generate income that enables farmers to improve and sustain their livelihood.

For most farmers in developing countries, soil fertility is one of the major problems facing agriculture. Chemical fertilizers are not always available; they are costly and cannot completely maintain the fragile equilibrium of

ecosystems. There is now a big and growing gap between the external nutrient inputs and the constant drain of nutrients from the soil, increasing the downward spiral of soil fertility loss.

This is where BNF can help. Although it is already making a large contribution in total nitrogen fixed globally, the need for BNF improvement and its use in agriculture has never been more urgent than it is today, especially for the vulnerable cropping systems in developing countries.

To face the growing challenges of soil fertility loss and the plateauing of legume productivity, it is necessary to develop and implement holistic approaches for sustainable crop improvement. For this, we must first promote systems of integrated nutrient management, crop rotation and intercropping with legumes. We can also apply modern biotechnology tools, especially those directed towards legumes as food for the poor. But this should always be combined with approaches to sustainable agriculture and greater farmer participation in production, post-production, and marketing. If properly applied, this will help resource-poor farmers to overcome the socioeconomic limit of access to chemical fertilizer, by providing alternative and complementary sources of nutrients. Increased production will then contribute to higher and more sustainable on-farm incomes and reduced poverty.

The new CP initiative is intended to open a new phase in international agricultural research and global partnership. The CGIAR has clearly defined the goals and established the process based on open calls for participation. The objective is *enhanced coordination to produce greater synergies between NARS, advanced research Institutes and IARCs, which should result in cost effectiveness of research and contribute significantly in technology transfer and capability building of NARS partners*. This initial planning workshop is mainly for stakeholders' involvement and participation in problem identification, research planning and implementation. We are also currently working to stimulate donor interest and commitment and prospecting the potential for attracting new funding to support a long-term effort of multidisciplinary research on legumes and BNF.

This initiative has all the necessary ingredients for success. I am also confident that with your help and hard work during this week we can all learn from each other, put our synergies into action and do our best to achieve the objectives of the workshop. The CP we target should not only generate knowledge about science and biotechnology, but aim to share this knowledge to increase the income of resource-poor farmers and improve their health and livelihood. The BNF program must contribute to solving the number one public health problem of the world – hunger.

Let me thank the management of INRA and ENSA Montpellier through Dr Benoit Jaillard for agreeing to host this meeting in this beautiful and historic city. I also thank the local organizers and especially Dr Drevon for

fostering the excellent teamwork with my ICRISAT colleagues headed by Dr Rachid Serraj that has made this workshop possible. I thank all colleagues from the Consultative Group for International Agricultural Research (CGIAR) and other international research centers who have joined hands with us in this challenge program initiative. I sincerely thank Dr Hardarson of the IAEA-FAO Joint Program for representing both international agencies here. IDRC and DFID, our friends from Canada and the United Kingdom, have generously accepted partial sponsorship for this workshop, and we thank them. The complete list of contributors is too long to read through here, so allow me to thank all of you for being with us today for this important meeting.

At this point, let me congratulate you all for attending this very relevant workshop – I wish you all success.

Thank you and good day.

Preface

Although most traditional cropping systems have always included a cereal-legume combination, legume production has not kept up with the dramatic increase in cereal production. The Green Revolution saw a phenomenal improvement in cereal grain production by selection of N-responsive crop varieties that produce more grain, and increased N in the form of added fertilizer. However, the high costs of N fertilizer production and its dependence on non-renewable energy sources, combined with the potential economic and environmental benefits of BNF systems, have prompted substantial research investments in BNF-legume technologies. In the current worldwide scenario of a nitrogen resource plateau and growing concern over possible adverse environmental effects of chemical fertilizers, as well as their cost for small-scale farmers in developing countries, it is essential to expand the use of the BNF technologies that offer the greatest environmental and economic benefits for each specific agroecosystem.

BNF is a significant element of any integrated soil fertility management strategy to reverse the degradation of cultivated lands. Particularly for the subsistence farmers who cannot afford inorganic fertilizers for staple crops, BNF is essential to improve food security, soil fertility and human livelihood. Important opportunities exist in the short, medium and long term for enhancing the global role of legumes in cropping systems.

In addition to their N_2-fixing capacity, legumes are extremely important in human and animal diets and significantly improve household health standards. Globally, they supply 33% of human protein, and some are important sources of oil. Other legumes provide unique phytochemicals, and some are sources of essential minerals like iron and zinc. Legume intensification within the farming systems also contributes to inter-seasonal food security, reduced stress, lower migration rates, and enhanced nutrition status of women and children.

In response to the initiative launched by CGIAR interim Science Council (iSC) for the implementation of global challenge programs for research on international agriculture, a concept note was submitted by ICRISAT for a CP on *Biological Nitrogen Fixation for Increased Crop Productivity, Enhanced Human Health and Sustained Soil Fertility*. The concept note was selected by the iSC, and given the approval for the development of a pre-proposal. This required

the holding of an international workshop and meeting of the main partners and stakeholders of the CP. The objectives of the workshop were to:

1. Review state of the art in the various aspects of Symbiotic Nitrogen Fixation: genomics, agronomy, participatory methods and socioeconomic limitations.
2. Constitute an Inter-Center Working Group on BNF (ICWG-BNF) and an international consortium for the Challenge Program.
3. Discuss research strategies and develop a pre-proposal for the CP-BNF.

The International Workshop on *Biological Nitrogen Fixation for Increased Crop Productivity, Enhanced Human Health and Sustained Soil Fertility* was held 10–14 June 2002 at ENSAM-INRA, Montpellier, France. The Workshop was co-organized by ICRISAT and INRA, and co-sponsored by IDRC and DFID.

The main goal of the workshop was to define research strategies for a Challenge Program initiative on Biological Nitrogen Fixation (CP-BNF), and develop a pre-proposal based on the concept note submitted and approved by the CGIAR iSC on January 2002.

More than 35 participants from various scientific disciplines, research institutions and from four different continents (Africa, Asia, Americas and Europe) attended the workshop (see list of participants), and worked synergistically to develop the framework for the CP-BNF initiative and pre-proposal. To further facilitate the interaction and scientific discussion between partners, after the workshop, an interactive website was launched, containing all CP information and a discussion forum (http://www.icrisat.org/bnf/Biological.htm). This collective effort has led to the development of a CP-BNF pre-proposal. This book, based on the proceedings of the workshop, takes a holistic approach to harnessing legume BNF technologies, starting from a socioeconomic perspective, and progressing to agronomic and genetic options, and bringing together innovative aspects and participatory research strategies.

I express my appreciation and gratitude to all the participants of the Workshop – session chairs, speakers, discussants, and those assisting with local arrangements. I also thank all the reviewers for their great help in improving the scientific quality of the papers. The Symposium and most invited speakers were generously supported by DFID and IDRC.

R. SERRAJ

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I

Overview of Legume SNF Research Achievements in IARCs

1

Harnessing the Potentials of BNF for Poor Farmers: Technological, Policy and Institutional Constraints and Research Needs

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ABSTRACT

Depletion of soil fertility and degradation of the production potential of agricultural lands are serious constraints to poverty alleviation and sustainable food security in many poor regions. Declining soil productivity in sub-Saharan Africa is also associated with low levels of fertilizer use, which is far below that of South Asia, Central and Latin America. High levels of risk and high fertilizer prices preclude smallholder farmers in many poor regions from accessing synthetic fertilizers. In regions like South-East Asia and the developed world, high levels of fertilizer use have increased the productivity of land and food production, but high energy and environmental costs associated with fertilizer use necessitate the search for alternative methods of soil fertility management. Nitrogen is the nutrient demanded in largest quantities by plants, and most expensive in the process of industrial production. Nitrogen is abundant in the atmosphere, but plants cannot directly utilize the elemental form available in the air. Biological nitrogen fixation occurs mainly through symbiotic association of legumes and some woody species with certain N_2-fixing microorganisms that convert elemental nitrogen into ammonia. Biological nitrogen fixation is therefore less costly and more sustainable. Scientific and technological progress has opened increasing opportunities for harnessing these potentials for the benefit of smallholder farmers. However, despite high investments in BNF research worldwide over the last few decades, benefits to developing country agriculture have been very limited. This paper assesses these limiting factors, with focus on technological, policy and institutional weaknesses and constraints, and suggests priority areas for future research.

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INTRODUCTION

In the face of increasing population growth and a concomitant decline in the area of land available for expansion of agriculture, many developing countries face the enormous challenge of increasing agricultural production. With the exception of sub-Saharan Africa where per capita food production continues to decline, the success of investments in agricultural research and the Green Revolution in Asia, however, led to substantial increase in per capita food production. Coupled with increasing population pressure and stubborn poverty, opportunities for intensification of agriculture are declining mainly due to degradation of the productive potential of soil, water and agro-biodiversity resources. At low population pressure, many production systems relied on fallowing agricultural land for replenishing soil fertility. Under the influence of high population pressure, such extensive systems of soil fertility management have almost disappeared in many parts of the developing world. In many highly populated regions, such as the highlands of East Africa, or in South Asia, the intensity of land use is already more than 100%. Moreover, increased intensity of land use is rarely accompanied by productivity-enhancing investments that protect the productive potential of the resource from declining over time. As will be discussed later, fertilizer use in many smallholder systems, especially in sub-Saharan Africa is still very low (Kelly et al. 1998). High levels of risk, poor markets, lack of access to credit, and low profitability of fertilizer – mainly due to high prices and low crop-fertilizer responses – are some factors that limit the demand for mineral fertilizers.

Biochemical and physical degradation of soil (referred to as soil degradation) ensues from exploitative land use practices. The extent and severity of land degradation in the developing countries is not sufficiently known. A recent worldwide initiative to estimate the extent of land degradation, the Global Assessment of Soil Degradation (GLASOD), indicates that in Africa, Asia, South America, and Central America 65%, 38%, 45% and 74% respectively of agricultural lands is affected to some degree (slight-to-extreme) by some type of degradation (Oldeman 1994). A study on nutrient balances in 38 countries of sub-Saharan Africa indicated a severe depletion of soil nutrients estimated at over 10 kg N ha^{-1}, 4 kg P_2O_5 ha^{-1}, and 10 kg K_2O ha^{-1} (Stoorvogel and Smaling 1990). Soil erosion by water and wind, depletion of soil nutrients, salinity, waterlogging, acidification and deforestation are the major agents of land degradation. Human-induced problems of soil degradation often arise from policy and institutional failures that lead to lack of incentives or lack of capability for sustainable use of resources. A recent IFPRI study provided a comprehensive review of the problem of land degradation at the global level, its economic costs, and policy and research priorities (Scherr 1999). Therefore, given the high rates of nutrient depletion

some scholars consider depletion of soil fertility, along with pest and disease incidence, as the most fundamental biophysical cause of low per capita food production in sub-Saharan Africa (Sanchez 2002). The equivalent fertilizer costs for replacing the annual net depletion of soil nutrients in sub-Saharan Africa is estimated at US$ 4 billion (ibid.). The actual economic cost of soil degradation, including the on-site productivity loss and the off-site economic and environmental externalities, is likely to be much larger.

Given the high levels of nutrient depletion and soil degradation in many smallholder systems, associated with high fertilizer prices that limit incentives for replenishing soil fertility, alternative nutrient management systems are urgently needed. Nitrogen is the soil nutrient element needed in greatest quantity by crops. A key component of the success of the Green Revolution in improving the yields of rice and wheat was the increased input of nitrogen fertilizer. Likewise, high yields of hybrid maize require abundant applications of nitrogen. The natural process of BNF can play a critical role in the achievement of cost-effective replacement of soil nitrogen and environmentally benign and more sustainable farming systems. BNF occurs through some species of microorganisms, which have the ability to convert atmospheric nitrogen into forms that are usable by plants and animals. Most of this occurs through some species of plant (legumes, ferns and some non-legume woody flowering plants), which establish a symbiotic association with microbes (like *Rhizobacea, Frankia, Azospirillium* and *Anabaena* species). Table 1 summarizes the BNF system. This process is very useful in agriculture, agro-forestry and forestry systems worldwide. Symbiotic nitrogen fixation in legumes allows them to grow well without the addition of fertilizer nitrogen. However, it may be necessary to apply phosphorus (P) and other deficient nutrients, as well as lime to alleviate soil acidity. Legumes are important sources of protein for many poor people in developing countries and serve as a source of feed in the developed world. The residual nitrogen in a system of crop rotation also

Table 1. The biological N_2 fixation system of symbiotic and free-living microbes.

Microbes	Nodulating parts	Relationship	Benefiting plants
Rhizobium	Roots, stems	Symbiotic	Legume crops and trees
Frankia	Roots	Symbiotic	Non-legume flowering plants (e.g. *Casuarina, Alnus*, etc.)
Azospirillum		Associative symbiosis	Grasses, sugarcane
Anabaena (Blue green algae)		Intracellular associative symbiosis	Azolla (aquatic fern) useful as green manure for paddy rice
Azotobacter		Free living[a]	Cereals, vegetables

[a]Because of its limited amount, N_2 fixation by the free-living microbes is considered to be less important in agriculture and forestry.

Source: Hardy (1993)

benefits cereal crops grown following legume crops, a practice very common in smallholder systems across the tropics.

Considering the high levels of soil degradation, and high economic and environmental costs of nitrogen fertilizer use, BNF seems to be a promising technology for small farmers in many developing regions. However, despite the enormous research investments over the last few decades, the development and application of BNF products and processes useful in developing country agriculture has been very limited (Hardy 1993, Bantilan and Johansen 1995, Singleton et al. 1997). This paper reviews the potential economic and environmental benefits and impacts of BNF systems on the livelihood of the poor, and the existing technological, policy and institutional constraints and weaknesses that limit full utilization of this potential. We also identify the key research areas from the socioeconomic and policy perspective that need to be addressed in order to develop economically viable and appropriate BNF technologies and to increase the access and utilization of these alternative options by smallholder farmers.

ECONOMIC AND ENVIRONMENTAL BENEFITS

Research investments in BNF systems have been justified on several accounts. Some of these benefits can be summarized as:

- economic (profitability)
- energy efficiency
- nutritional gains for the poor
- environmental quality
- agricultural sustainability

Economic Benefits

Increased use of BNF systems is expected to reduce the need for industrially (non-biologically) fixed fertilizer nitrogen. The reduced demand for fertilizer N will then lower costs of production without affecting crop yields, thereby providing the small farmer the opportunity to raise returns to land and labor. Returns to land and labor may not decline even if yields fall slightly so long as the cost savings from N fertilizer use are significant. However, a yield decline will have implications for food and nutritional security in areas that already have supply shortfalls. If crop yields increase due to BNF and costs decline, producers may still benefit from reduced costs of production even if market prices decline. The fall in consumer prices will also help net buyers and urban consumers. In the context of developing countries, this has not yet been realized or even properly demonstrated (see next section).

Table 2. The yield response of tropical legumes to rhizobial inoculation under differing levels of management.

Legume crops	Total number of trials[a]	Significant yield response[b] (% of trials)	
		Traditional management	Improved management
Mung bean	40	70	68
Soybean	40	65	65
Black gram	15	53	60
Lentil	27	48	41
Groundnut	26	50	46
Chickpea	31	48	55
Cowpea	9	56	11
Common bean	10	10	30
Pigeonpea	8	13	13

[a]Distributed across different countries, covering 20 countries for all legume experiments.

[b]Significant responses are given as yield increments greater than one standard deviation (>1 SD) from inoculation compared to uninoculated controls. These values are based on the synthesis of results given by Singleton et al. (1992) for experiments run by the University of Hawaii: Nitrogen Fixation by Tropical Agricultural Legumes (NiFTAL).

The extent to which BNF can replace (even partly) fertilizer N and lower costs of production is not clearly established under farmers' conditions. Results from one study, which synthesized the responsiveness of legume crops to rhizobial inoculation, are presented in Table 2. This data does not however provide the grain and biomass yield responses to rhizobial inoculation.

Table 3 shows the approximate amount of N_2 fixed and the share of plant nitrogen derived from BNF. These results indicate a varying level of response by crop type, which in turn is influenced by several factors. Some

Table 3. Average amounts of N fixed by various legume species and their microsymbionts.

Legume species	Approximate amount of N_2 fixed (kg ha^{-1})	N requirement met from N_2 fixation[a] (%)
Leucaena	325	-
Lucerne	250	-
Pigeonpea	220	52-88
Cowpea	210	32-74
Mung bean	200	89-90
Stylo	125	-
Soybean	110	70-80
Groundnut	100	54-78
Chickpea	102	60-80
Common bean	50	37-68

[a]These values are only selected estimates. The share of plant N derived from BNF varies enormously depending on the genotype of the same crop and the environment in which it is grown (Giller, 2001).
Source: Montanez (2000) quoting different sources and Giller (2001).

crops like the common bean seem to have lower responsiveness to inoculation. It is important to compare the economics of returns from BNF technologies with that of using alternative inputs. The small farmer will have the economic incentive to adopt BNF technologies only when the expected returns are higher than the use of alternative inputs (e.g., organic and inorganic fertilizers). In systems where fertilizer N is rarely used on legumes, these responses may be substantial given the low unit costs of inoculants. One hundred grams of inoculant are usually sufficient for 20,000 to 100,000 seeds, making the costs per plant inoculated very low. This therefore offers enormous cost advantages with respect to the use of N fertilizer in using potent inoculants.

Unfortunately, as will be explored in the next section, because of technical and socioeconomic weaknesses, this potential remains unrealized in developing country agriculture.

Energy Efficiency

Non-biological N_2 fixation in fertilizer industries is an energy-intensive production process involving the use of non-renewable energy resources, mainly natural gas, petroleum and coal. In the 1970s, concern over rapidly increasing prices and declining stocks of fossil fuels focused attention on BNF research (NRC 1994). Of the three major nutrients (N, P and K), N production requires the highest amount of energy both as raw material and as fuel for processing. Nitrogen fertilizer accounted for about 87% of the total energy demand of the global fertilizer economy in 1990 (Bumb and Baanante 1996). Phosphorus and potassium are mostly derived from mined phosphate and rock and potash ores and therefore require only modest quantities of energy for processing. Nitrogen fertilizer products, like urea and ammonium nitrate, are produced from ammonia (NH_3). Natural gas, petroleum, naphtha and coal are the hydrocarbons most commonly used in the production of ammonia and N fertilizer. When supplies of natural gas become limited, industry will undoubtedly return to coal.

The efficiency of energy use in N fertilizer production varies widely across countries depending on the source of energy and the efficiency of organization in the process of production and maintenance. Bumb and Baanante (1996) provide country specific data for energy consumption in ammonia production, which varies from 26-28 British Thermal Units (BTUs) in modern plants in the developed regions to 50-60 BTUs in small plants in India and China. They argue that despite the high-energy demand for N fertilizer production, the energy demand of the fertilizer sector in 1990 accounted for only 2% of the global energy consumption. This share is expected to decline further due to expected improvements in energy efficiency. However, unlike industrial fixation of N, the energy requirements of BNF,

although large, are met by renewable sources such as plant-synthesized carbohydrates using solar energy rather than that from nonrenewable fossil fuels such as natural gas.

Nutrition and Food Security for the Poor

In many countries, human nutrition is highly dependent on grain legumes as major sources of protein, especially for the poor, who cannot afford animal products like meat and eggs. It is estimated that about 20% of food protein worldwide is derived from legumes. The major consumption centers of legumes are in the former Soviet Union, South America, Central America, Mexico, India, Turkey, Greece and North Africa. Some available data indicate that the dietary use of legumes is quantitatively in the following order (Agostini and Khan 1986):

- dry bean (*Phaseolus vulgaris*)
- dry pea (*Pisum sativum*)
- chickpea (*Cicer arietinum*)
- broad bean (Vicia faba)
- pigeonpea (*Cajanus cajan*)
- cowpea (*Vigna unguiculata*)
- lentil (*Lens culinaris*)

Groundnuts (*Arachis hypogaea*) and soybean (*Glycine max*) are dominant sources of cooking oil and protein for humans and livestock. They are also major food sources in some regions. The amino acid components of leguminous seed proteins commonly show deficiency in cysteine and methionine, but when consumed in combination with cereal protein offer a good nutritional balance. Thus, the legume complements amino acid deficiencies in cereal grains. Moreover, the importance of legumes in animal feed should not be overlooked. Alfalfa (*Medicago sativa*), clovers (*Trifolium* spp.), stylosanthes (*Stylosanthes* spp.), desmodium (*Desmodium* spp.), and other forages are grown extensively (NRC 1994).

Environmental Quality

A number of significant environmental concerns have been outlined to justify the search for alternatives to chemically fixed nitrogen fertilizer: it affects the balance of the global nitrogen cycle, pollutes groundwater, increases the risk of chemical spills, and increases atmospheric nitrous oxide (N_2O), a potent greenhouse gas. Crops use fertilizer nitrogen inefficiently; the plant utilizes not more than 50% of applied N fertilizer. Of the other 50% or more, some is stored in soil organic matter, where it becomes available to subsequent crops, some is converted back to atmospheric nitrogen through denitrification, and

some is leached and pollutes the groundwater as nitrate (NO_3). In contrast, the legume crop assimilates all the biologically fixed nitrogen (i.e., BNF is 100% efficient).

Nitrous oxide, along with carbon dioxide (CO_2), methane (CH_4), and chlorofluorocarbons (CFCs), is a greenhouse gas that traps reflected sunlight and may cause global warming. The energy reflectivity per mole of N_2O is about 180 times that of CO_2. Denitrification of nitrate produces about 90% N_2 gas and 10% N_2O. The production of nitrogen fertilizer by industrial nitrogen fixation not only depletes finite reserves of fossil fuels, but also generates large quantities of CO_2, the principal greenhouse gas. Thus, increased use of fertilizer nitrogen may contribute substantially to the potential for global warming, and early replacement with BNF is desirable (NRC 1994).

Sustainability of Agriculture

As discussed above, soil degradation and low fertility of tropical soils is a major constraint to increased food and fiber production. Mineral fertilizers have been used in increasing quantities to offset the problems of poor soil fertility and boost agricultural production. Many small farmers, however, still lack the possibilities for using fertilizers. Where heavy use of fertilizer N is encouraged through subsidies, it is associated with high environmental problems. Many would also argue that without fertilizer N, which contributed to the success of the Green Revolution, social and environmental costs would have been much higher. However, the future seems to hold a better option; BNF offers an economically attractive and ecologically sound means of reducing external N input and improving the quality of soil resources. The need for fertilizers, production of which depends on non-sustainable energy sources, can be reduced by N supplied through BNF in the field, produced through more sustainable biological processes using a renewable source of energy (solar energy). If successfully developed for marginal environments, BNF could also contribute to better soil cover and build up of soil organic matter, which promotes water infiltration, protects soils from erosion, and enhances carbon sequestration.

TECHNOLOGICAL AND SOCIOECONOMIC CONSTRAINTS

The increasing recognition of the potential economic and environmental benefits from developing BNF systems has prompted research investments estimated at US$ 50-100 million per annum worldwide in the last three decades (Hardy 1993). Some of these investments have already generated products and processes that found their application mainly in industrial agriculture in the USA, Canada and Australia. Many of the potential returns

are, however, expected to accrue only in the long term (over 5-10 years). Recent advances in molecular genetics and DNA fingerprinting have opened new possibilities for harnessing the potentials of BNF systems. While increasing the contribution of BNF systems in food production worldwide remains to be a significant scientific challenge, utilizing the existing potentials in the developing regions requires a holistic approach to research and development and a conducive socioeconomic and policy environment for utilization of these alternative systems of N fertilization.

Technological Constraints

The necessary condition for a new technology to be attractive to farmers is that it has to provide higher relative returns to land and labor compared to the best competing alternative option. In some cases, adoption of a new option may also occur not for profitability reasons, but because of taste preferences or because of its better ability to withstand production risk, especially when risk markets are imperfect. In this case, the benefit derived from adopting the new option would be higher than the old option even if average returns over time may be the same. Only when such tangible economic incentives exist would rational farmers consider adopting a technology. When such incentives are believed to exist and adoption fails to occur, other factors called adoption constraints can limit the potential impact of the new technology. These adoption constraints include the influence of imperfect markets (input and credit delivery mechanisms, lack of markets for products, etc.), incomplete property rights, weak institutions, and distortive policies (e.g. subsidies, taxes, quotas) that tip the balance towards the old (existing) option.

In the case of BNF systems, there are a number of technical problems that limit the application of the technology at a wider level in the developing world. These limitations include:

- Crop specificity of the microorganisms
- Low amounts of N_2 fixation achieved in the field (environmental limitations)
- Poor competitiveness of added rhizobia with native species
- Poor stability – loss of viability of the inoculants under physical and climatic stress during production, storage and field use
- Failure to develop formulations that provide pre-inoculated seeds
- Inhibition due to fertilizer N application (lack of complementarity)
- Limitation to legumes (not cereals) reduces potential impacts on livelihoods and the environment

The rhizobia have a high specificity for host legumes and soil types, which implies the need to develop *Rhizobium* strains suitable for specific crop types in a given environment. This requires heavy investments and technical skills – scarce resources in developing countries – for production and dissemination of the technology. It hinders a farmer growing different legume types from buying one general formulation for use in all legumes that he grows. This lowers the flexibility and perceived competitiveness of BNF systems with respect to the use of nitrogen fertilizers. Broader host range (or use of promiscuous legume varieties) would simplify production, distribution, marketing, and grower use of inoculants.

The amount of N_2 fixed by legume crops varies substantially depending on various factors, host genotype, *Rhizobium* efficiency, soil and climatic conditions. Table 3 summarizes the approximate level of N_2 fixed by different legume crops. The common bean has the lowest N_2-fixing ability, while Leucaena, a protein-rich agro-forestry species, fixes the highest amount per ha. The level of N_2 fixation also falls when the crops are grown in intercrops due to reduced legume density or competition for resources from non-legume intercrops. Zero tillage and minimum tillage also seem to increase N_2 fixation (Van Kessel and Hartely 2000).

Since the desired type of N_2-fixing microsymbiont may not exist in the required amounts in a given soil, inoculation with an appropriate strain suited for a specific crop and soil conditions is often required. The inoculation technology comes in different forms; powder or granular forms are common. The powder form is applied directly to the seeds before planting. The granular form allows application directly into the furrow with the seed. For the powder form about 210 g is needed to treat seeds planted per ha. The granular form requires 5-30 kg ha^{-1} depending on row width and planting density. None of the methods available now allow embodying the technology as part of the seeds. Pre-inoculated seeds, if made possible, would greatly simplify marketing, transport and distribution of the inoculants. The major problems with inoculants are their poor competitiveness with local strains; sensitivity to climatic and other stresses limiting their viability and number; and problems of packing, transport and storage until end-use on the farm (Smith 1987, Bantilan and Johansen 1995). Without refrigeration, the live microbial culture fast loses its potency, making use of the inoculant technology a difficult option under smallholder farming conditions in the tropics (Smith 1987, Bantilan and Johansen 1995, Singleton et al. 1997, Montanez 2000).

The high sensitivity of the inoculum to temperature and other climatic stresses drastically reduces the most probable number (MPN) of effective microbes, thereby reducing the yield responses to inoculation at the farm level. This makes the technology almost inapplicable under smallholder conditions, and creates a bad perception on the part of the end user about the potentials of BNF systems. The *Rhizobium* inoculant could be one example of

a technology that has been pushed to users before the technical and institutional setups in many developing countries were ready for its widespread use. The initial failures are very likely to affect adoption of the technology in the future when viable options are developed. Hence, despite some efforts to promote biological fertilization technologies in India and other developing regions, the effort has been frustrated by low viability of the existing inoculation technologies and lack of enforceable quality control systems (Bantilan and Johansen 1995, Singleton et al. 1997). Therefore, along with reducing host-specificity, improving competitiveness and efficiency, packaging and formulating inoculants to withstand adverse effects of high temperature and other stresses, and improved availability of good quality inoculants in the market are *essential* first steps to make BNF useful to smallholder farmers in the tropics.

The inhibition of BNF by N fertilizer application also creates difficulties for use of the technology in certain production systems. Ideally, a farmer would like to combine some amount of fertilizers (N, P, and K) with BNF in a given production activity. If fertilizer use reduces the efficiency of BNF, complementary and synergistic input use is impossible. This may be even more important when farmers grow legumes in intercropping systems with cereals (as is common in India) and N fertilizers cannot be separately applied for the cereals. The limitation of BNF systems to the legumes and certain woody species also substantially reduces the potential impacts of BNF. The economic and environmental benefits and impacts on the livelihood of the poor in developing countries are likely to remain limited unless the technology can also be applied to major food crops (cereals). The already low yields of legume crops, low demand for legume products, and socioeconomic and policy distortions (e.g., subsidies and quotas) reduce the relative returns and the potential impacts of the technology now limited to legume crops.

Further, considering the complexities inherent in the agricultural production environment and its influence on the effectiveness of BNF systems, as Hall and Clark (1995) argue, scientifically-derived technologies cannot cope alone with the scale of the problem unless farmers themselves are recognized as a source of innovations whose knowledge is complementary to that of the formal research process. Lack of such a holistic perspective and the inability of the formal research system to deal with local complexity and diversity have contributed to the inherent weaknesses of the process of technology development, promotion and utilization.

Policy and Institutional Constraints

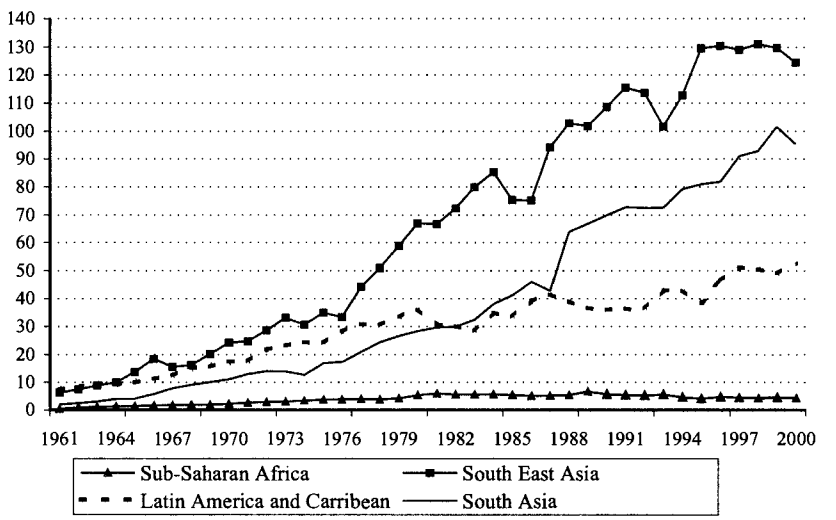
Apart from the technical problems that limit the economic benefits of the inoculant technology, a wide range of policy and institutional failures, including poor market access and weak quality control and extension systems

have been blamed for lack of success of the BNF system in developing countries (Singleton et al. 1997, Alam 2000). The major socioeconomic and policy issues, frequently mentioned as reasons for low demand, and hence limited impact, of BNF systems are:

- Fertilizer subsidies
- Price support (subsidies) and higher relative cereal prices
- Poor market development for legume products
- Poor inoculant market development
- Moral hazard and adverse selection in inoculant markets
- Weak extension systems and lack of awareness and insufficient demonstration to farmers

Fertilizer subsidies

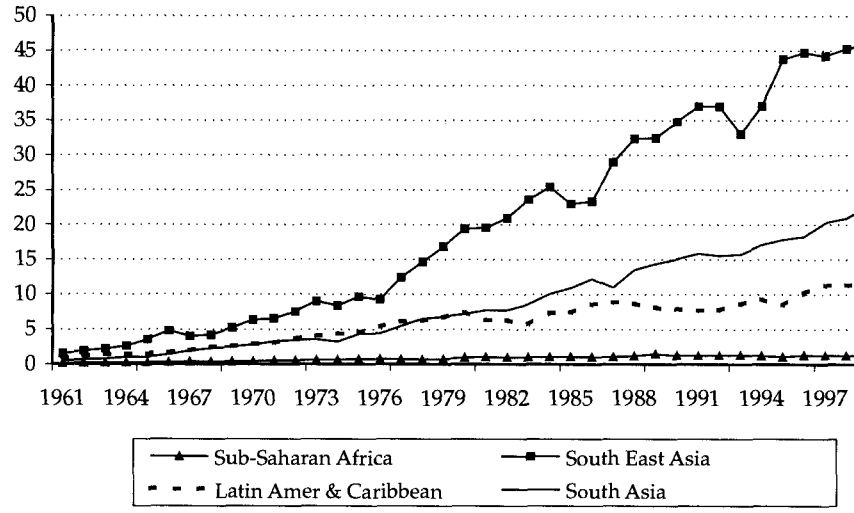
Fertilizers have played a prominent role in increasing the supply of food in poorer regions and helped in averting famines and starvation in many parts of the world. Many governments in the past have therefore consciously encouraged increased fertilizer use through product price supports and input subsidies. The regional fertilizer use data presented in Fig. 1 shows the lowest level of fertilizer use per hectare in sub-Saharan Africa.



Source: Compiled based on FAO Statistical Database.

Figure 1. Regional trends in fertilizer use (plant nutrients) in kg ha^{-1}, 1961-2000.

Both applications per hectare and absolute levels of consumption are lowest for the sub-Saharan region (Fig. 2).



Source: Compiled based on FAO Statistical Database.

Figure 2. Regional trends in fertilizer consumption (plant nutrients) in million tons, 1961-2000.

Hence, despite some subsidies in the past and declining per capita food production, fertilizer consumption in sub-Saharan Africa has shown little change over decades (Bumb and Baanante 1996, Kelly et al. 1998). In 1970, sub-Saharan Africa used about 5 kg ha^{-1}, which has shown only a marginal increase in 1997 to 9 kg ha^{-1} (Kelly et al. 1998). In terms of actual plant nutrients, fertilizer use in the region grew from about 1.5 kg ha^{-1} in the 1960s to 4.8 kg ha^{-1} in the 1990s. As shown in Figs. 1 and 2, this is dramatically different from the pattern that emerges in other developing regions. This low level of consumption is due to low returns from intensification and high risk, which justifies the need for alternative systems of soil fertility management. FAO projections to 2006 indicate that the demand for fertilizer N in Africa is expected to grow by about 2.5% per year (FAO 2001). Given the very low levels of current consumption, this will not however change the overall picture of low levels of use of fertilizers in the region.

As has been argued, subsidies for synthetic fertilizers can lower or obliterate the economic incentives for adopting biological fertilization systems. Coupled with advantages of chemical fertilizers in packaging, transport, storage and application, the lower (subsidized) input prices for N fertilizers could dampen the benefits from BNF systems. There is a shortage

of data to support the empirical significance of this claim. It is difficult to separate whether the low demand for *Rhizobium* inoculants is due to their general ineffectiveness at the farm level or is due to fertilizer subsidies. In India biofertilizers are also subsidized (Alam 2000). To the extent that fertilizer subsidies discourage individual farmers from adopting more sustainable fertilization systems, one can argue for policy interventions to internalize the possible policy failure. This is because private economic agents do not consider the environmental benefits accruing to society from adoption of more sustainable systems like BNF. This causes a classic externality problem, which reduces the level of use of BNF to below what is socially optimal. Such intervention would require more research to estimate the social economic and environmental benefits from BNF systems.

Thanks to the structural adjustment and economic liberalization policies of the 1980s and 1990s, many countries in sub-Saharan Africa have phased out fertilizer subsidies, and indeed, all kinds of agricultural subsidies. Before the subsidies were phased out, many countries followed pan-territorial pricing polices for fertilizers, an egalitarian policy which tried to ensure a more equitable delivery of fertilizers to farmers across the country. Following the devaluation of the overvalued domestic currency and the phased removal of subsidies, fertilizer prices skyrocketed in many countries, thereby further limiting the demand for fertilizers in many parts of Africa. The improvements in efficiency of procurement and marketing of fertilizer, which were expected to lower prices, did not materialize in many cases. Hence, farmers, located in remote locations with low market access and infrastructure, pay two to three times the prices paid at the center or near the port of entry (Sanchez 2002). Unfortunately, these farmers also earn the lowest prices for their products, thereby further lowering the incentives for fertilizer use. Under suitable conditions, these kinds of marginalized farmers are likely to benefit from alternative and cheaper fertilizers. These farmers have for centuries been dependent on traditional soil-fertility enhancing methods like fallowing systems, cereal-legume rotations, and use of farmyard manure. However, many of these options are increasingly becoming difficult due to high population densities and the high opportunity cost of agricultural by-products for use as fuel wood (dung and crop residues) or for feeding livestock (crop by-products).

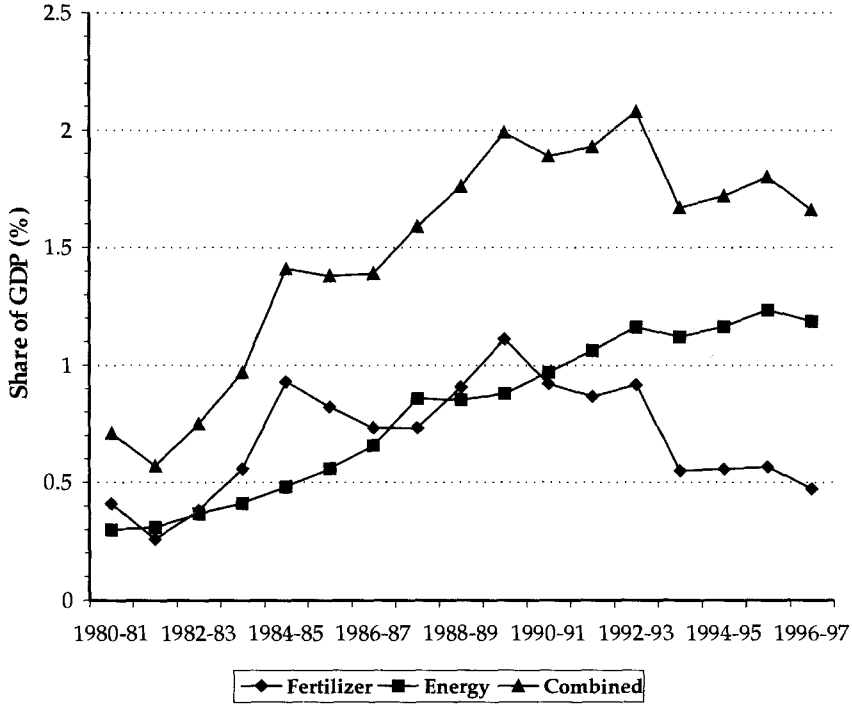
Unlike in sub-Saharan Africa, many countries in Asia, including China, India, Indonesia and Saudi Arabia, subsidize mineral fertilizers (Bumb and Baanante 1996). The prices that sub-Saharan farmers pay compared to those in Asia is presented in Table 4.

In the Asian countries, fertilizer subsidies account for a significant percentage of the government budget contributing to high fiscal subsidies, low efficiency of fertilizer use (often less than 30-40%) and discouraging investments in alternative sources of soil fertility management. Figure 3 presents the dynamics of fertilizer subsidies in India.

Table 4. Fertilizer prices (US$/metric ton) paid by farmers in Africa and Asia, 1991/92.

Country	Urea	DAP	MOP
Africa			
- Morocco	249	257	188
- Senegal	-	365	-
- Zambia	256	-	487
- Zimbabwe	359	-	232
Asia			
- Bangladesh	126	140	136
- India	118	181	66
- Indonesia	110	-	141
- Nepal	120	176	68
- Pakistan	162	201	-

DAP: Diammonium phosphate, MOP: Muriate of Potash
Source: Bumb and Baanante (1996)



Source: Using data from Gulati and Narayanan (2000).

Figure 3. Dynamics of fertilizer and power subsidies in India.

In the last two decades, the fertilizer and energy subsidies in India have together accounted for 0.6-2% (the average is 1.5%) of the gross domestic product (Gulati and Narayanan 2000). Not much is known about the

macroeconomic and environmental effect of such pricing policies and impacts on alternative technology development investments. More research is needed in this area to understand the potential interlinkages and effects on economic and environmental systems.

One should also add that the N fertilizer substitution effects of BNF systems are not as high as suggested by some authors. As presented in Table 5, the share of fertilizer currently used on legumes in developing and developed countries, is only 2.8% and 4.6%, respectively. Much of this is likely to be accounted for by P and potassium fertilizers. The respective share of cereals is 53.3% and 65.8%, while the balance goes to vegetable crops and forages (Bumb and Baanante 1996). This indicates that the share of total fertilizer used on legume crops is quite limited.

Table 5. Relative shares (%) of fertilizer use by region and crop, 1989-91.

Region	Cereals	Roots and tubers	Legumes[a]	Other crops[b]	Hay and forage
North America	70.3	1.4	6.1	9.6	12.6
Western Europe	44.3	3.3	1.3	21.2	29.9
Eastern Europe	41.8	8.3	1.2	18.2	30.5
Eurasia	47.1	8.6	0.4	11.5	32.4
Oceania	43.8	1.4	3.5	22.5	28.7
Africa	59.2	4.1	3.3	29.6	3.8
-North	58.4	4.2	3.9	33.5	...
-Sub-Saharan	63.8	5.2	3.5	27.2	0.3
-South	55.2	2.7	2.0	26.5	13.6
Latin America	41.4	4.1	12.1	40.3	2.1
-Central	57.6	2.0	1.6	36.8	2.0
-South	33.8	5.0	17.1	42.0	2.1
Asia	68.7	5.5	3.5	21.5	0.8
-East	68.3	7.8	3.9	19.1	0.9
-South	71.7	1.3	2.9	24.1	...
-West	58.1	2.9	3.1	32.2	3.7
World	59.3	4.6	3.7	19.8	12.6
Developed countries	53.3	4.0	2.8	15.9	24.0
Developing countries	65.8	5.3	4.6	23.8	0.5

[a]Includes pulses and soybeans

[b]Includes fruits and vegetables, oilseeds, tree crops, sugarcane/sugarbeet, cotton, tobacco and others

Source: Bumb and Baanante (1996) quoting various sources.

Hence, the major social benefit from BNF systems limited to legume crops is much less the gain in environmental quality than the increased profitability of these crops resulting from cost savings on reduced fertilizer use or possible improvements in yields. Any possibilities for extending BNF systems to cereals, where a lion's share of the N fertilizer is used, could however be expected to provide tremendous economic and environmental benefits globally.

Cereal price support (subsidies)

If government policy intentionally rewards cereals through price supports or other policies that encourage increasing acreage or production of cereals, the relative returns to pulses will be low. The effect of such a policy, which is similar to a tax on legume output or acreage, will create incentives to decrease legume production and increase cereal production. It would be interesting to investigate how quotas or agricultural price policies affect sub-sectoral investments between cereals, legumes and other crops and adoption of new technologies. We lack data on the relative level of price supports for cereals and legumes across countries.

Poor markets for legume products

Under smallholder farming, many of the legumes are produced in smaller quantities mainly for home consumption rather than for markets. Since legumes are often used in crop rotations mainly for soil fertility improvement and as yields are very low, there is not much marketable surplus. This implies that the market for legumes could potentially be highly imperfect, in such a way that price, supply and demand information is either missing or greatly incomplete. The market is highly disorganized, the number of dealers is insufficient and competition is limited. If this is true in some parts of the developing countries, setting up processing industries that add value to primary products or expanding the demand for legume crops or commodities could be difficult. If BNF is likely to have an impact on the livelihood outcomes for the poor, expanding market opportunities for legumes and stimulating the demand for these products should be part of the research and development strategy.

Imperfect inoculant markets

The size of the world market for rhizobial inoculants was projected to be worth only US$ 50 million by the year 2000. About US$ 20 million of this was found in the United States alone (Singleton et al. 1997). As was discussed earlier, inoculants need to be very specific to a given crop and production system. This requires developing internal capacity for production, storage and marketing these products. However, the small markets that may exist in many developing countries (especially where the total acreage under legumes is limited) are unlikely to attract the required investments for inoculant production and marketing facilities. The low viability of inoculants under climatic stresses and the high host-specificity of the microbes tend to increase the risk of investment. This is also likely to discourage the participation of the private sector in developing products and processes for inoculants. Some exceptions are China and India where the domestic market is large due to

extensive legume production. How best to encourage the involvement of the private sector and develop a more viable inoculant industry in developing countries is an important area for research. The success of this will have a significant impact on the success of the BNF systems.

Moral hazard and adverse selection problems

Production and marketing of high-quality inoculant technologies is very critical for encouraging adoption of BNF systems. One major problem, which now affects the growth of the industry, is failure to verify the viability of the inoculants at the point of purchase. This is mainly due to poor quality control and non-enforceable standards that distort the market incentives of the inoculant sellers, a serious institutional problem that threatens to stifle the industry. When information about quality of a product is segmented and only one of the dealers (in this case the seller) knows for sure the inherent quality of the product, the classic problem of imperfect information known as adverse selection occurs. The high-quality inoculum available in the market is indistinguishable from the bad quality products, because there is no mechanism to verify quality on the spot. In this case, dealers of better quality inoculum are crowded out of the market, because they cannot fetch higher prices for their quality products. The producers and dealers of inferior inoculum take advantage of the environment of imperfect information, creating a situation of moral hazard. Singleton et al. (1997) report that about half of the inoculant samples tested from 12 developing countries had less than the threshold number of rhizobia ($1x10^8$ rhizobia per g) needed for viability of the inoculant. Developing enforceable standards, quality control methods and regulatory mechanisms would help improve the production and delivery of quality inoculum and minimize the problems of adverse selection and moral hazard in marketing rhizobial inoculums.

Weak extension and delivery systems

Many developing countries have yet to build the domestic capacity for production, marketing and dissemination of live bacterial cultures for soil fertility management. This requires heavy investments in training and revamping the old extension system, which may be biased towards the known fertilizer and seed technologies. Some governments have tried to push BNF systems through subsidies and quotas. Alam (2000) gives some examples in India where the government gives quotas to extension agents for selling inoculant products at subsidized prices and how lack of confidence in the technologies on the part of the extension system is affecting outreach for these technologies. In an effort to maintain their credibility with farmers, the extension agents in some parts of India are reported to have paid from their own salaries to meet the selling quotas for biofertilizers set by the government

(Alam 2000). This is a problem of technology, which is pushed without sufficient guarantees about its viability and effectiveness under farmers' conditions. Only few smallholder farmers in the developing countries (mainly in India, China and Thailand) are now exposed to these technologies. The poor effectiveness of the inoculum and its low quality undermine the extension and dissemination efforts. As more viable BNF technologies are developed, on-farm participatory technology verification trials and demonstration systems would be needed. Concerted education, skill enhancement, promotion of the new technology, better understanding of its limitations and potentials, application systems, etc. are important elements of a capacity building strategy that will encourage a shift to more sustainable systems of agricultural intensification.

RESEARCH NEEDS

The review and synthesis of the potentials, opportunities and limiting factors for harnessing biological nitrogen fertilization systems for the benefit of the poor farmers in developing countries presented heretofore suggest a number of priority issues for BNF research in the area of socioeconomics and policy. These can be categorized under the following themes:

- Technologies
- Agricultural development and price policies
- Markets
- Institutions

Available information indicates that current applications of BNF in developing countries are generally constrained by the poor effectiveness and competitiveness of the technology compared to other alternatives for soil fertility management. This requires concerted effort for improving the efficiency and stability of the technology, especially to make it more suitable under stressful and constrained production conditions of small farmers. Research should also develop a simple and convenient form of the product (including packaging and storage systems) for easier delivery through available supply channels without the technology losing its potency. Since the BNF technology is lower in volume and weight than synthetic fertilizers, distribution and marketing of the product would be much easier if made available as pre-inoculated seeds. Along with application of molecular methods on the host-microbe system, understanding the influence on BNF of improved soil fertility management systems and agronomic practices that increase the demand for nitrogen needs to be properly examined. On the economic and policy side, more work needs to be done to assess the economic and environmental net-gains to society from BNF and understanding the influence of agricultural input-output pricing policies for the development

of alternative and more sustainable soil fertility management systems. On the institutional side, harnessing the potentials of BNF in developing countries would require development of markets for legume products, addressing the problems of imperfect information in marketing quality BNF products, and developing institutional capacity (research, extension and regulatory standards for quality control and application) to tackle the weaknesses related to marketing, storage, delivery and farm-level deployment of BNF technologies.

A summary of the socioeconomic and policy related research issues is provided in the box below.

Research issues for BNF.

1. Technologies

- Increase competitiveness of added inoculants with native microbes
- Improve the level of N_2 fixation and crop yield responses to inoculation
- Reduce the host specificity of the inoculants or develop more promiscuous legume varieties
- Selection of N-tolerant symbiosis to enhance complementary use of mineral fertilizer N with BNF
- Develop plants and inoculants that perform well under stress (high temperature, desiccation, oxygen sensitivity, etc)
- Develop improved agronomic systems for enhancing BNF and extend benefits to cereals through rotations and intercropping

2. Socioeconomic and policy issues

- Understand the effects of pricing and marketing policies for fertilizers and competing crops on the demand and profitability of legumes
- Estimate the minimum benefits from BNF systems that would create incentives to farmers to switch from using mineral fertilizer to biofertilizers
- Identify distributional (welfare) and environmental benefits from BNF systems
- Identify new policy options and incentive systems to promote BNF systems

3. Markets

- Increase commercialization of legumes through improved market access

(Contd.)

(Contd.)

- Improve the competitiveness of grain legumes through increasing yields and quality
- Stimulate the demand for legume products (through processing for value addition, exports, etc.)
- Identify new market niches and develop mechanisms for linking farmers to markets
- Develop new ways for enhancing the utilization of legume crops
- Improve availability of viable inoculants to the smallholder farmers

4. Institutions

- Enhance farmer participation in technology development and evaluation
- Develop the national and local capacity for production, storage and dissemination of effective BNF technologies
- Support and encourage private sector participation in development of viable BNF products and processes
- Develop the capacity for regulation and quality control of inoculants
- Develop institution arrangements for enhancing awareness and demonstration of potentials of biological N fertilization systems to poor smallholder farmers

PRIORITY SETTING

In a world of uncertainties and resource constraints, the relative risk and rate of returns from a given investment are major considerations that influence the level of investment in a given portfolio. Although knowledge of the relative risk among competing investment options is often very difficult to acquire, subjective assessments of future options and technological capabilities may help rank the alternatives areas of research investments. The difficulties in setting such priorities are related to difficulty in making reliable assessment concerning the relative expected returns from research investments. This is because consumer preferences often change and alternative products or technologies that serve as substitutes may be developed. To the extent that a reliable assessment of the risk of success and expected returns from a given research can be made, a decision rule for research priority setting could be developed. Perhaps lessons from the last few decades of research in BNF may help develop priorities for future investment in this area. An improved version of a priority-setting guide developed by Hardy (1993) is depicted in Fig. 4.

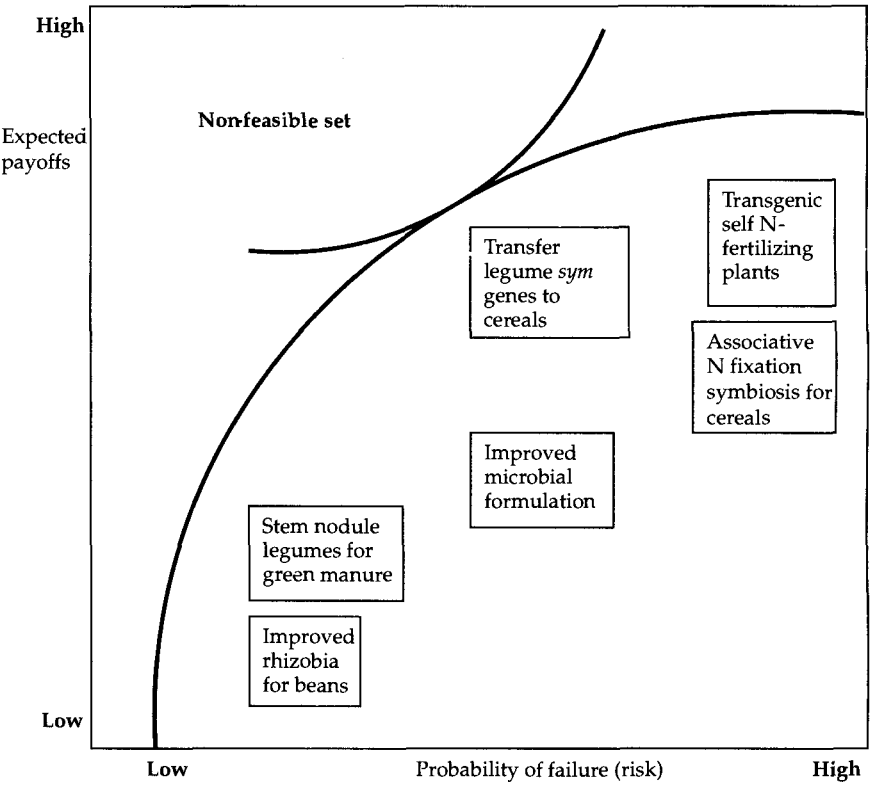


Figure 4. Decision guide for priority setting in BNF research.

Within the feasible region, the different areas of research bring varying expected net returns at different periods. Private sector investors may focus on combinations with lower risk and higher short-term expected net returns. Public sector investors may however be willing to take some risks and invest in areas that bring higher returns in the short-to-medium term. In theory, the most efficient research options are all those located along the curve; but the differences in the payoff periods make the efficiency mapping less important.

SUMMARY AND IMPLICATIONS

Nitrogen is the soil nutrient element needed in greatest quantity by crops. Many studies have documented the critical role N fertilizer has played for the success of the Green Revolution in improving the yields of rice and wheat. Likewise, high yields of hybrid maize require abundant applications of nitrogen. Hence, synthetic N fertilizer use has grown from 3 million to over

90 million tons in the last 50 years, more than twice the estimated 40 million tons of N_2 fixed by crops worldwide. This increase occurred in both developed and developing countries. The current annual worldwide expenditure for fertilizer nitrogen exceeds $20 billion. Projections for the next three years indicate that the global demand for N fertilizer is expected to grow by 1.7% per annum. The regions with deficit balances that need to import N fertilizers include North America, South Asia, East Asia, Western Europe and Oceania. The regions with surplus balance that could export N fertilizers include Eastern and Central Europe, Central Asia and Latin America. Africa is also expected to have limited surplus, but will need to import some as the demand exceeds supply around 2006 (FAO 2001). The high costs of N fertilizer production, dependence on non-renewable energy sources, and the potential economic and environmental benefits from BNF systems continue to encourage research investments in this area. The low competitiveness of existing BNF technologies vis-à-vis N fertilizer use also continues to discourage the usefulness of new products to farmers.

There are more than 13,000 described species of legumes. Of the approximately 3,000 species examined, more than 90% form root nodules. Because few have so far been exploited for food, there is the prospect that the utilization of legumes could be expanded substantially (NRC 1994). The increasing opportunities that exist in the post-genomics era in developing N_2-fixing capabilities in major cereal crops also open prospects for developing ecologically more sustainable and economically viable agricultural systems in many areas now suffering from poverty and degradation of the resource base. Research in BNF systems has therefore a tremendous hidden potential for alleviating global poverty, food insecurity and environmental degradation. Along with the scientific advances of the past decades, the CGIAR centers, which have already accumulated enormous data and knowledge on BNF systems in various tropical legumes, can play a pivotal role in spearheading research in this area in the developing world. There are good prospects for developing alternative biological N fertilization systems that may partly or totally eliminate the demand for N fertilizer in growing legume crops. Given the high prices (emanating from poor market access and high transaction costs) and high levels of risk in drought-prone rainfed systems that limit the demand for N fertilizers in many poor countries, development of effective BNF systems applicable to low input agriculture and agro-forestry can be expected to confer very significant economic and environmental benefits.

Despite significant breakthroughs in developing BNF systems in the past few years, the benefits from such improvements were not captured in developing country agriculture mainly due to problems in transferring benefits observed in laboratories to farmers' socioeconomic and biophysical growing conditions. This has limited the economic gains from adopting *Rhizobium* inoculants and made BNF less competitive compared to the use of N

fertilizers. Many small farmers in developing countries have consciously used the capabilities of legumes in fixing N_2 for centuries. Even when the net benefits from the BNF technologies are positive, many small farmers are largely unaware of improved BNF options. However, much remains to be done in improving and refining the BNF technology, especially in developing processes and products usable in smallholder agriculture. This requires strong partnerships with farmers and the private sector. Agricultural policies that subsidize N fertilizers and/or cereal food crops and reduce the relative returns from legume crops, along with poor institutional capabilities that exist in developing countries, also limit harnessing the promising potentials of BNF for the benefit of the poor. This study has assessed these technological, policy and institutional limiting factors and proposes areas that require further research. Priority areas for research include increasing the efficiency, stability and competitiveness of the BNF technology (compared to synthetic fertilizer N); adapting the technology to suit the conditions of smallholder farming; and understanding the influence of the structure of markets, agricultural input-output price policies and institutional arrangements (e.g., quality control, legislation, research and extension) for the development of alternative and more sustainable systems of soil fertility management.

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Increasing the Role of Legumes in Smallholder Farming Systems – The Future Challenge

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ABSTRACT

This paper has a threefold purpose: (1) To share some of the experiences the ICRISAT Southern Africa Team has had in Farmer Participatory Research and the development of Soil Fertility Management options; (2) To highlight some of the major concerns in the promotion of BNF and legumes from a farming systems perspective; and (3) To share some of the hopes and aspirations of colleagues who work in Africa and cannot be present at this meeting. From this debate and experiences within ICRISAT six major lessons have been learnt about the differential adoption and targeting of alternative soil water and nutrient management options to differently resourced households:

Soil fertility gradients are the norm with P deficiencies typically increasing as the distance from the homestead increases. Legumes play an important role in smallholder farming systems and there is room for improvements and increasing the land area planted to these crops. No enabling policies encourage smallholder farmer investment in soil fertility management. Undeveloped input and output markets limit legume intensification. Legume intensification needs to target poor households for food for home consumption and wealthier households for cash income through producing marketable surpluses. Small quantities of fertilizer, manure-fertilizer and legume-fertilizer combinations have a high payoff and supplying inorganic fertilizers in small packs reduces the liquidity constraint faced by many households and enhances returns to investment. To address these issues it is essential that a framework be developed to analyze the trade-offs in African smallholder farming systems and provide a platform that enables knowledge sharing between disciplines and across national boundaries.

One such approach currently being developed is NUANCES - Nutrient Use in Animal and Cropping systems – Efficiency and Scales.

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INTRODUCTION

The agroecosystem of the African continent is extremely diverse, reflecting not only the physical geography and climatic variations, but also the socioeconomic and cultural diversity of its inhabitants. Over the last 10 years, the five African subregions have experienced sharply divergent trends in productivity. West Africa and North Africa have seen fairly solid annual growth of about 3-5% within the agricultural sector. Central Africa has seen solid growth in some commodities (cereals 4.0 %, cocoa 2.6%) and a decline or flat output for others (coffee 5.4%, oil crops 0.8%). This is in sharp contrast to Southern and Eastern Africa, where per capita grain production continues to decline, despite the adoption of new crop varieties (FAO 1999). In the past 20 years Zimbabwe, Kenya, Tanzania and Malawi have changed from net cereal grain exporters to grain importers. Poverty is worsening in the rural areas of each of these nations, compounded by the HIV/AIDS pandemic and economic structural readjustment programs (Devereux and Maxwell 2001).

Most of the increase in per capita production in Africa over the past 30 years or so has been achieved by bringing more land under cultivation. Today, only about 7% of Africa's total land area (about 150 million ha) is devoted to crop production. But given climatic constraints, poor soils and a very uneven distribution of water resources, only another 30 million ha of unused land can be potentially be brought under cultivation without further jeopardizing the environment (Twomlow and van der Mere 1998). The combination of population growth, low incomes and food insecurity encourages destruction of the agricultural resource base through soil mining and farming practices that lead to soil erosion (Fischer and Heilig 1997). The time horizon of many rural households is from one season to the next, as meeting short-term household food requirements negates longer term planning for resource conservation and/or improvement (Devereux and Maxwell 2001). The problem is that incentives to pursue environmentally sustainable practices are commonly lower than incentives to simply extract natural resources. The value of an additional dollar of output today is worth far more to most small-scale farmers than the value of much larger production levels in the distant future. Many developed countries have resolved this problem by paying farmers either to take land out of production or to adopt more sustainable practices. Unfortunately, few developing countries have the capacity to make similar investments. In fact the majority of Africa's poorest and most food-insecure households live in the semi-arid tropics (SAT). To survive in a harsh and variable environment, they pursue a range of livelihood strategies. Different households pursue different development paths. But almost all seek to diversify their income sources and investment strategies as a means to reduce risk and if possible, respond to rapidly changing market conditions.

Therefore, in reality, any significant increases in productivity within the SAT will have to come from an intensification of the existing systems. As agriculture is both extensified and intensified, traditional management practices are insufficient to maintain soil fertility, let alone increase system productivity. This situation is exacerbated as men leave the farms in search of off-farm employment, leaving women to raise families and run farms on a deteriorating resource base with a reduced pool of household labor. Recent ICRISAT surveys in the drier regions of Malawi and Zimbabwe suggest that in some rural areas up to 50% of rural households are headed by women (Ahmed et al. 1997, Freeman 2000, Rohrbach 2000).

Unfortunately, increasing productivity in the SAT of Africa has proved an intractable problem for traditional farming research programs, mainly because of poor uptake of soil, water and nutrient management technologies by smallholder farmers. Low adoption rates of available soil management options in the SAT are due to technologies that are inappropriate to farmer conditions (e.g. high labor and fertility input demand) and attitudes towards risk, ineffective input/output markets and extension, and a policy environment that discourages investment interventions (Dixon et al. 1989, Sanders et al. 1996, Barbier 1997, Scoones and Toulmin 1999, Devereux and Maxwell 2001, Ryan and Spencer 2001). To help overcome these constraints, it is necessary for rural households to devise and implement more appropriate soil, crop, water, livestock, and tree management options, as well as finding options for improving markets, policies and institutions. Research input is required to help identify opportunities within rural household livelihood strategies that facilitate adoption, rather than carrying out research work on uniform experimental plots with few if any production constraints, unless they be the focus of the research. The purpose of this paper is threefold:

1. To share some of the experiences the ICRISAT Southern Africa Team has had in Farmer Participatory Research and the development of Integrated Soil Fertility Management (ISFM) options;
2. To highlight some of the major concerns in the promotion of Biological Nitrogen Fixation (BNF) and legumes from a farming system's perspective; and
3. To share some of the hopes and aspirations of colleagues who work in Africa and would like to contribute to the debate on legume intensification and the associated benefits from BNF.

THE CONTRIBUTION OF LEGUMES TO SMALLHOLDER FARMING SYSTEMS OF THE AFRICAN SAT

It is now time to focus on the role of legumes within the farming systems, particularly of the poorer SAT regions, to achieve the potential contribution

of crop and livestock production systems to household food security, improved nutrition, income-generating opportunities and sustainability. Recent trends in agricultural production and consumption point to increasing livestock production, even in SAT (FAO 1999). The question that is then important is what role legumes might play as this trend evolves and what opportunities exist to exploit their food, feed and N_2 fixation potential to develop more sustainable mixed farming systems. Despite the efforts of the Tropical Soil Biological Fertility Programme (TSBF), the African Association for Biological Nitrogen Fixation (AABNF) and their partners from other IARCS, ARIs and NARES within Africa, legumes are still erroneously seen as subsistence crops grown by the poorest sectors of the population, and all too often ignored by research and development. Consequently, legumes are under-utilized species in many of the farming systems of the African SAT, rarely achieving their full potential in terms of food and/or non- food products (AABNF 2001, Giller 2001). Recent ICRISAT surveys (Rohrbach 2000) in southern Zimbabwe clearly show that there is

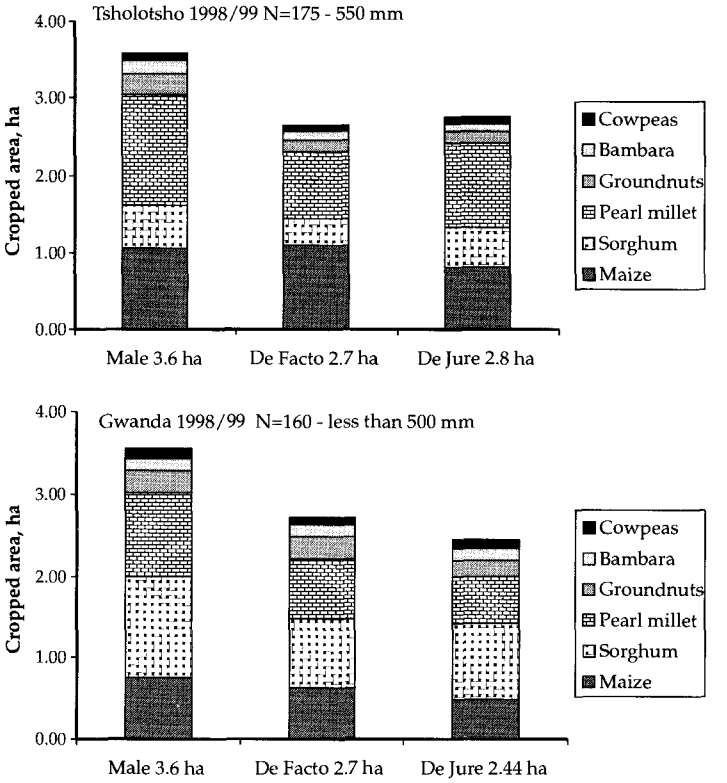

Figure 1. Current area of smallholder land devoted to legumes for two areas in southern Zimbabwe. The marital status and gender of the household head influences the crops grown.

considerable potential for expansion of their use for improvement of small-scale farmer poverty alleviation, food security and soil fertility enhancement. Figure 1 shows the current area of smallholder land devoted to legumes and other crops in two areas of Zimbabwe for three classes of household: Male headed; *De facto* – husband away working; *De jure* – household headed by a single women (divorced or widowed).

The need to ask questions that are both pertinent and relevant to the needs of rural households goes without saying. But sadly the experience over many decades has been that too often scientists have asked questions related to the scientific aspects of the work without much consideration of the real users (Scoones and Thompson 1994). The existence of non-adopted and non-adapted blanket fertilizer recommendations, after decades of investment in trials of all sorts in country after country, is witness to such a process of inappropriate field research (Scoones and Toulmin 1999). In fact, it is not just in fertilizer research, but in many branches of agricultural science (Pretty 2002), that the scientists continue to ask the standard questions relating to maximal yield, rather than the questions that would enable the end user to achieve optimal yields in relation to the household's resource constraints and marital status and gender of the head of household (Fig. 1).

As Mike Swift observed (1998, p59, 16th World Congress of Soil Science)

> *Soil science has been brilliantly informed by reductionist physics and chemistry, poorly informed by ecology and geography, and largely uninformed by the social sciences.*

RURAL LIVELIHOODS – IDENTIFYING AVENUES OF INTERVENTION FOR ISFM AND BNF

The productivity of soil is extremely important to all rural households, and soil fertility is often a key constraint in areas where crop production has been practiced continuously for a long time (Scoones 2001). Soil fertility management is a complex interaction and a challenging problem that is often not apparent, involving the interaction of soil, water, organic matter, macro- and micronutrients, and microorganisms, with high levels of variability over space and time. Gradients in soil fertility are the norm rather than the exception as the distance from the homestead increases (Tilahun Amede (CIAT/AHI) pers comm., Twomlow et al. 2000). Successful soil management demands a lot of practical knowledge of soil processes, many of which are invisible. When the soil fertility problems are clearly observable, farmers can readily establish cause-effect relationships. Indeed, farmers have a good understanding of soil fertility effects on plant vitality and growth: a fertile soil produces healthy plants that are resistant to pests and diseases, and produce high yields.

Some key points from southern Africa:

- The majority of rural communities have their own criteria for classifying their soils, such as moisture holding capacity, ease of cultivation, inherent fertility and which crops grow best where within the landholding, with many households actively managing their soils in ways that build upon these criteria (Fig. 2 - adapted from Twomlow et al. 2000).

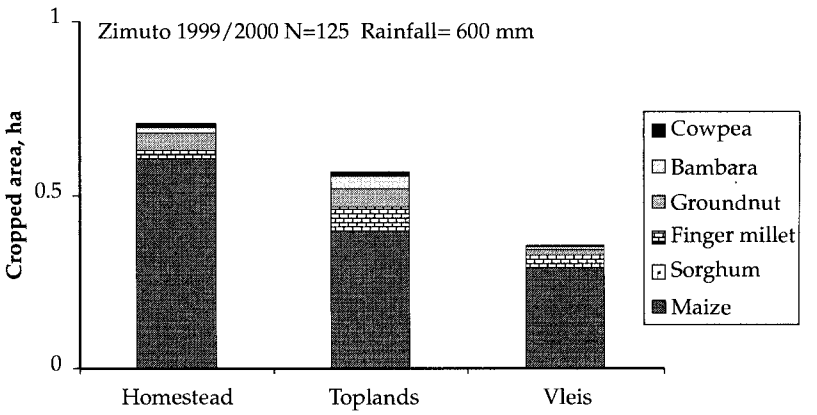

Figure 2. Current area of smallholder land devoted to legumes on different field types in Zimuto, Zimbabwe.

- Africa's smallholder farming systems are highly diverse and diversity is an important feature of smallholder systems on all scales. At household level, this diversity will be managed through different land use practices, choice of crops (Fig. 2) and level of inputs. At a national scale this diversity is seen in the differential development of areas considered high and low potential, infrastructural investment and ease of access to important markets.
- Farming systems are dynamic, responding to a range of internal and external factors that mean that no two households will follow exactly the same soil fertility management pathway (Fig. 3, adapted from Twomlow et al. 2000).
- A household's management of soil nutrients and crops grown is influenced by a number of social and economic factors. These factors include access to land, livestock, labor, credit and markets – but also knowledge and social institutions that may provide access to some resources. Figure 4, a simplified farming system, shows the flow of nutrients around the fields of a well-resourced household, including a flow of crop residues from a neighboring poorer household that does not possess livestock (Giller 2002).

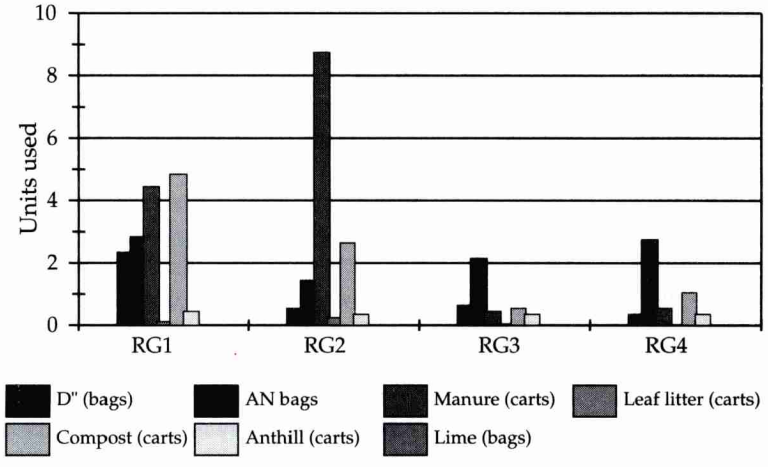

Figure 3. Variation in soil fertility amendments for homestead fields in Zimuto, Zimbabwe for 4 different household resource categories.

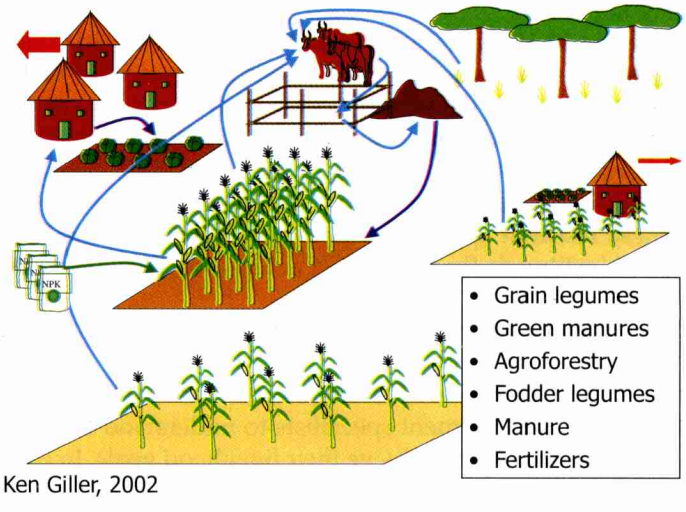

Figure 4. A simplified farming system showing the flow of nutrients around a well-resourced household, including a flow of crop residues from the poorer resourced household. The shading of the different fields reflects soil fertility status and the intensification of production. Darker soils are fertile and more intensively used. Lighter soils reflect a decline in soil fertility and more extensive use (adapted from Giller 2002, pers comm.).

The key points raised above are not unique to southern Africa, but have commonalities with many of the smallholder farming systems in all of sub-Saharan Africa. For example, in east Africa legumes are grown in the most fertile corner of the farm for two chief reasons. First, the productivity of outfields

is low and the returns to growing a cereal crop are much greater than the return expected from a legume on these infertile soils. If a legume is grown on these outfields, it may be in a fertile pocket, such as an old anthill, or as an intercrop with a fertilized maize or coffee crop. Second, the most important crops, such as sweet potatoes and legume grain crops (in terms of food security), are grown in the fields closest to the homestead, as farmers believe that growing legumes in rotation with these crops will not exhaust the soils, unlike cereals such as sorghum and maize or Teff (an Ethiopian staple cereal). However, there are very clear niches for legumes in the upper highlands (>2400 m above sea level), where conditions limit the farmers' current choice to a few legumes. For example in the upper Ethiopian Highlands, the system is dominated solely by barley, and there are only a few legumes adapted to the cold harsh conditions, such as vetches and clovers. Consequently, farmers frequently leave up to 50% of their land as a fallow, the only option available to them to restore soil fertility. Similar challenges may occur in other mountainous regions of the Andes, Nepal and Tibet where farmers might welcome a wider range of leguminous crops that could contribute to soil fertility and have some feed/food value. In this instance, getting frost-resistant food legumes could be much more challenging than BNF.

Over the past 10-15 years many researchers have attempted to take account of this diversity in smallholder systems through the application of the 'Farmer Participatory Research Paradigm' that recognizes farmers needs and aspirations, and the role extension and development institutions should play in linking farmers with markets (Ashby et al. 1987, Chambers et al. 1989, van Veldhuizen et al. 1997, Guijt 1998, Hagmann et al. 1997). More recently these research approaches and tools have been combined into a 'Sustainable Livelihoods Framework' (Carney 1998, Scoones 1998).

THE SUSTAINABLE LIVELIHOODS FRAMEWORK

In its simplest form the Sustainable Livelihoods Framework (SLF) is a tool to help research and development specialists to understand how people use resources at their disposal to achieve their livelihood goals. In essence it:

- Provides a checklist of issues that research and development specialists need to consider
- Assesses the potential contribution to livelihood sustainability made by existing activities
- Highlights what influences what
- Emphasizes the multiple interactions that affect the livelihoods of rural people
- Plans new research development initiatives

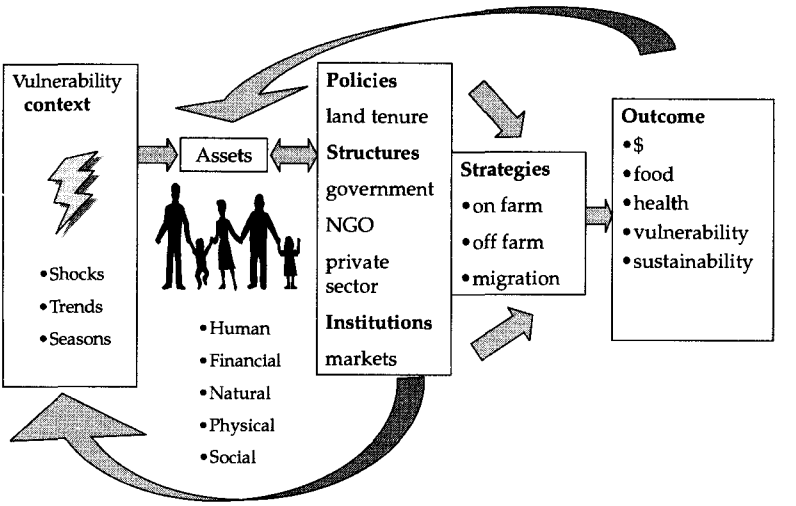

Figure 5. The sustainable livelihoods approach (adapted from Tanner 2001).

In addition SLF helps us to think holistically about:

- The things that the very poor might be very vulnerable to
- The assets and resources that help them to thrive and survive
- The policies and institutions that impact upon livelihoods
- How the poor respond to threats and opportunities
- What sort of outcomes people aspire to

The livelihood approach allows us to look at a wide range of influences within a single frame of analysis, as is shown in Fig. 5.

Understanding the various components of this approach requires the consideration of a range of facts/questions that affect each component:

The Vulnerability Context

This reflects the external environment within which people exist on a day-to-day basis and can be summarized in terms of:

- *Trends* – population growth, pressure on resource availability, economic environment, governance and technologies available
- *Shocks* – illness (i.e. HIV/AIDS pandemic), natural disasters (floods, droughts, earthquakes), economic conflict, crop/livestock interactions with pests and diseases, policy changes
- *Seasons* – price fluctuations, production, health, off-farm employment opportunities

Assets

Livelihood assets are various types of capital available to people and households from both rural and urban locations (including research and development specialists who are part of transforming structures and processes), from which they can fashion a livelihood. They include:

- *Natural capital* – land, water, wildlife, biodiversity, environment
- *Financial capital* – savings, credit, remittances, pensions
- *Physical capital* – transport, shelter, water, energy, communications (road networks)
- *Human capital* – skills, knowledge and information, ability to work, health
- *Social capital* – networks, groups, trust, access to institutions.

The capitals become resources for livelihood strategies as they are put to use, and can be sometimes substituted or traded off against each other, or capitalized upon to generate future resources (e.g. Fig. 6). The asset endowments of different groups are thus highly significant in determining the range of livelihood options open to households, and the importance of

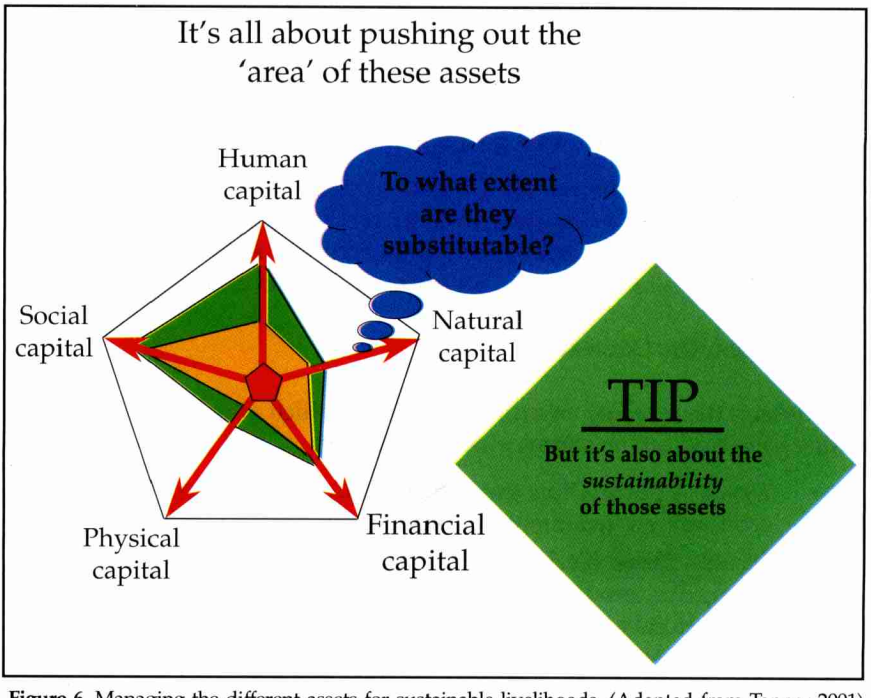
It's all about pushing out the 'area' of these assets
Human capital
To what extent are they substitutable?
Social capital
Natural capital
Physical capital
Financial capital
TIP
But it's also about the sustainability of those assets

Figure 6. Managing the different assets for sustainable livelihoods. (Adapted from Tanner 2001).

having capital to generate further capital can often explain much of the observed social differentiation in livelihood strategies.

Institutions

Institutional arrangements (in the broadest sense, the rules and norms which govern individual and group behavior) and organizational forms play a critical role, since they determine the access of individuals and households to the five types of livelihood asset. Institutions range from customary and local rule systems, determining for example how one group of resource uses may relate to another on a day-to-day basis (e.g. access to grazing or water), to formal laws and administrative procedures governing the use of forests. Institutions range from legal structures put in place by the government to social arrangements backed by moral pressures or sanctions. Many of these institutional systems overlap, creating competing jurisdiction over a household's access to resources, within individuals and groups manipulating the various institutions to construct their own strategies for resource access.

Within these more formalized structures are the institutionalized norms of masculine and feminine behavior that shape and influence the livelihoods of different households and their access to resources (see Fig. 1)

Livelihood Strategies – What do People do?

The majority of households pursue a combination of strategies together or sequentially to meet their livelihood objectives and be summarized as:

- Natural resource-based
- Non-natural resource-based/off-farm activities
- Migration/remittances
- Intensification vs. diversification
- Straddling between natural resource-based and non-natural resource-based activities
- Competition for household labor pools
- Short-term vs. long-term.

Recent survey work in Zimbabwe for three different locations along the 500 to 600 mm isohyets illustrates the influence that household location and ethnic grouping has on the income-generating activities undertaken (Fig. 7, adapted from Twomlow 2001).

These strategies result in the livelihood outcomes households aspire to.

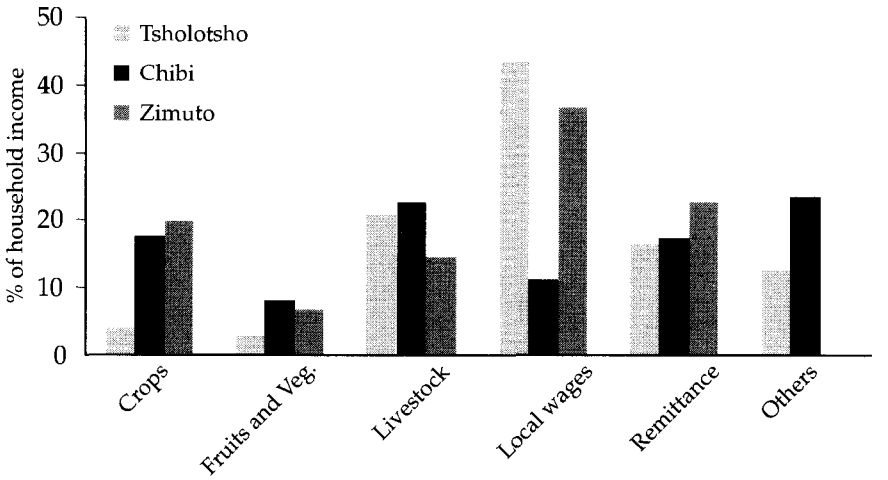

Figure 7. Income-generating livelihood strategies for three locations in Zimbabwe along the 500 to 600 mm isohyets. Tsholotsho - large land holdings, extensive with off-farm employment opportunities. Chibi - smaller land holdings and little opportunity for off-farm employment. Zimuto - smallest land holdings, intensive and opportunities for off-farm employment.

Livelihood Outcomes - What are People Seeking to Achieve?

- Reduced vulnerability
- Improved food security
- Increased well-being
- More income
- More sustainable use of the natural resource base.

SLF CONTRIBUTION TO INCREASING IMPACTS OF BNF RESEARCH IN SMALLHOLDER SYSTEMS

At the end of the day the SLF approach aims to provide a framework that facilitates research and development specialists to see development from a rural or urban household perspective, rather than their own, and helps develop synergies and understanding. It has major implications for the way BNF proponents should move forward to meet their goals as:

- specialists in our respective disciplines
- at national, subregional, regional and global levels
- with all stakeholders and
- with the donor community

and assist them in developing some key questions around ISFM and where BNF might contribute to SLF. For example:

- What are the current agricultural management practices being undertaken?
    - Are they the same for all households?
    - Are the same practices and crops grown on all fields? If no, why not?
    - Where are legumes currently grown?
    - Can legumes be grown in all fields?
    - Where do households currently target their fertility amendments?
- What are different strategies for ISFM being employed by different resource groups?
    - Cash cropping with inorganic fertilizers
    - Organic gardening around the homestead
    - Intercropping legumes with other crops
- What incentives are required to facilitate purchase of inputs – nutrients, seed, labor, transport?
- Do markets exist for proposed interventions and can they be transported at an economic cost?
- What institutions and organizations influence the ability of different resource groups to gain access to new knowledge, tools, seeds and fertilizers required for both improving livelihoods and sustainable soil management. In Malawi, a major thrust on legume intensification faltered because the seed delivery systems were not developed at the same time as the ISFM legume based technologies (Twomlow et al. 2001).

CONCLUSIONS

From this debate and experiences within ICRISAT six major lessons have been learned about the differential adoption and targeting of alternative soil water and nutrient management options to **differently-resourced households**:

- Soil fertility gradients are the norm with P deficiencies typically increasing as the distance from the homestead increases. This influences where a household will grow its legumes.
- Legumes play an important role in smallholder farming systems and there is room for improvements and increasing the land area planted to these crops.

- No enabling policies exist that encourage smallholder farmer investment in soil fertility management.
- Undeveloped input and output markets limit legume intensification, particularly seed supply.
- Legume intensification needs to target poor households for food for home consumption and wealthier households for cash income through producing marketable surpluses.
- Small quantities of fertilizer, manure-fertilizer and legume-fertilizer combinations have a high payoff and supplying inorganic fertilizers in small packs reduces the liquidity constraint faced by many households and enhances returns to investment.

To address these issues it is essential that a framework be developed to analyze the trade-offs in African smallholder farming systems and provide a platform that enables knowledge sharing between disciplines and across national boundaries (for example see Fig. 8, Tilahun Amede, CIAT/AHI, pers comm.).

A new approach currently being developed to promote both organic and inorganic soil fertility amendments is NUANCES - Nutrient Use in Animal and Cropping systems – Efficiency and Scales (Box 1 - Giller, pers comm.).

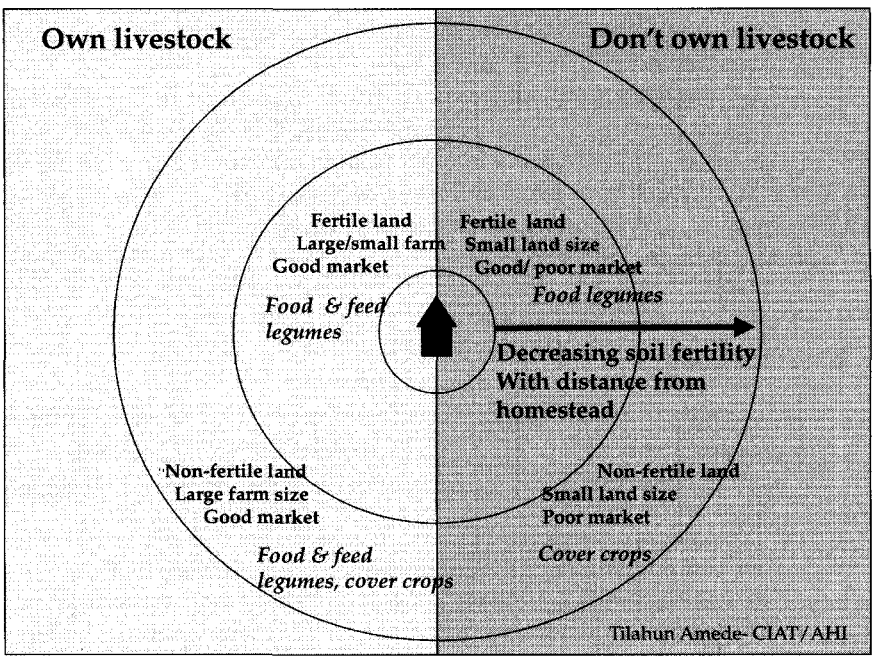

Figure 8. Conceptual framework to identify where legumes may be introduced into existing farming systems in East Africa. (Suggested interventions are in italics).

FINAL THOUGHTS

Great increases in BNF at the plot scale are possible by relieving simple restrictions such as nutrient limitations, availability of inoculums, new and better-adapted varieties with improved disease resistance, and the introduction of improved agronomic practices. Relief of these restrictions may appear simple, but in reality are some of the major challenges that need to be faced. Major advances in BNF can be made by the introduction of new legume varieties to different areas, retention of the residues, breeding and strain selection. However, none of this will occur without market development. In the longer term genetic engineering of host plants and development of BNF in new host species may be long-term goals, they do not offer a solution within the time horizon of most smallholder farmers!

Box 1. The NUANCES Framework (Giller 2002, pers comm.)

The NUANCES framework

A framework for iterative modeling and experimentation

- **Soil-crop models**
- **Livestock models**
- **Nutrient balances**
- **Optimization models**

Goals

- **Targeting of scarce nutrient resources of different qualities**
- **Assessment and intervention**
- **Trade-offs for nutrients, labor, cash and environment**
- **Design of more productive, sustainable systems**

Key features

- **Scaling in time AND space**
- **Need for simplicity**
- **Modular structure**
- **Use of expert (scientist and farmer) knowledge**
- **Decisions on principal drivers**

Links to policy?

- **Direct links to local policy**
- **Basis for evaluation at higher scales**

KEY ISSUES FOR CONSIDERATION IN DEVELOPING FUTURE RESEARCH AGENDAS ON BNF

- Glossary of terms to help communication between agronomists, breeders, microbiologists and biotechnologists
- Characterization of the macro/meso and micro issues that influence adoption/adaptation of legumes in the different agroecoregions of the developing world
- Agree on a series of sequential goals

- Raise existing yields in smallholder systems from 500 kg ha^{-1} to 1000 kg ha^{-1}
- Increase the proportion of land area cropped to legumes by developing market opportunities
- Multiple hypotheses must be linked to achieve impact
- Need to understand the advantages and disadvantages of promiscuous and nonpromiscuous legumes and the associated soil fauna
- BNF is an integrated part of the whole farming system and cannot be viewed in isolation
- What policy development is required to ensure the bio-safety of the agroecosystem
- What is required to develop sustainable input supply systems of seeds and inoculums

The success of future BNF research depends crucially on the ongoing learning processes between natural and social sciences, farmers and policymakers.

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3

Biotechnology-based Contributions to Enhancing Legume Productivity in Resource-poor Areas

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ABSTRACT

Throughout history, civilization has depended on cereal-legume cropping systems, but in recent times, cereal production has dramatically increased while that of legumes has not. Increasing cultivation of legumes will ameliorate environmental degradation, reduce depletion of non-renewable resources and provide adequate nitrogen for sustainable agriculture. Enhanced legume utilization and yield will increase protein production and agricultural productivity and reduce resource-poor farmers' dependency on expensive chemical fertilizers. For achieving optimal levels of symbiotic nitrogen fixation (SNF), medium-term impacts are possible through biotech-assisted germplasm enhancement, and long-term impacts through bioinformatics-assisted germplasm gene mining and utilization. Recent technological developments in the field of genomics have provided new tools to understand and manipulate the structure and function of entire genomes in a way never before possible. Developments in model species will fuel rapid advances in the lesser-studied tropical legumes while also providing genetic stocks and genetic populations of substantial value for more detailed studies in tropical legume crops. Success in decreasing the sensitivity of SNF to environmental constraints and thereby facilitating the expression of its full potential benefit requires attention to both partners of the legume-*Rhizobium* symbiosis, and exploitation of the new tools and methodologies of legume genomics. Comparisons between the legumes using genomic tools may show why certain legumes are better at N_2 fixation than others. This paper proposes a systemic collaboration of disciplines from the very beginning of the innovation pipeline that will facilitate the understanding and effective manipulation of a range of traits affecting legume productivity under marginal cropping systems. Legume genomics and marker-assisted breeding, strategies for legume marker development, comparative legume genomics, reverse genetics, DNA chip technology, functional genomics and serial analysis of gene expression have been discussed with a view to identifying genotypes that offer substantially higher

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SNF efficiencies under marginal cropping environments. Through a combination of comparative and functional genomics, bioinformatics and marker-assisted breeding it should now be possible to identify and manipulate the key loci conferring increased and stable legume productivity in resource-poor environments.

INTRODUCTION TO THE MOLECULAR BIOLOGY OF LEGUMES AND SYMBIOTIC NITROGEN FIXATION (SNF)

There is an urgent need for dramatic increases in food productivity in the tropics to keep pace with the rapidly increasing population in these areas. Similarly, there is a critical need for diversification of cropping systems to enhance the nutritional well being of poor people across the tropics. An increasing proportion of the world's poor is associated with population-dense regions of the semi-arid tropics especially in South Asia and sub-Saharan Africa, where pressure on soil fertility and water availability compound the problems for crop production. Sadly, it is these same areas that will be most detrimentally affected by climate change.

The Green Revolution of the 1960s was associated with dramatic increases in productivity of crops grown under high input systems. This success has led to a rise in the use of synthetic nitrogen from 3 million to 80 million tons over the last 40 years with parallel increases in the developing and developed countries (FAO 2002). This equates to an annual global expenditure on fertilizer in excess of $20 billion, with a similar expenditure on pesticide applications. Fertilizer production, being based on natural gas or natural fossil resources, is highly energy intensive and thus prices are likely to steadily increase during the coming years.

Throughout history, agriculture has depended on cropping systems that combined a nitrogen-consuming cereal with a nitrogen-fixing legume. In recent history cereal productivity has dramatically increased whilst legume yields have neared a plateau, stagnated or even reduced. This has resulted in unbalanced cereal/legume global production (Fig. 1), and thus in higher and unsustainable dependence on N chemical fertilizer inputs (FAO 2001). There is clearly an urgent need to enhance legume utilization and yield to increase protein production and the productivity of agriculture as a whole.

Legumes account for just 15% of arable farming land worldwide yet provide 66% of human nutrition needs in subsistence farming communities of the developing world. Symbiotic nitrogen fixation (SNF) from these crops contributes approximately 17 million metric tons of atmospheric nitrogen worth $8 billion to agriculture in USA alone (FAO 2001). Overall, SNF reduces resource-poor farmers' dependency on expensive chemical fertilizers, improves the soil and water quality and reduces their dependence on petroleum-based products. Increasing cultivation of legumes will be required to ameliorate environmental degradation, reduce depletion of non-renewable resources and provide adequate nitrogen for sustainable agriculture.

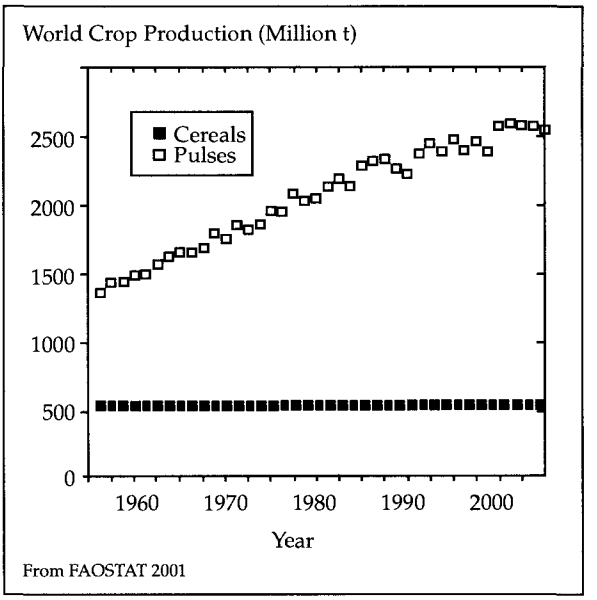

Figure 1. Cereal/legume global production.

Plants typically assimilate mineral nitrogen in the form of nitrate (Butz and Jackson 1977) or ammonium (Gazzarini et al. 1999). SNF occurs only in microorganisms that possess the enzyme nitrogenase. Contrary to free-living, N_2-fixing bacteria, many microorganisms fix N_2 by forming symbiotic relationships with legumes, lichens and some woody plants. The bacteria collectively known as rhizobia (virtually exclusive to legumes) and actinomycetes (non-leguminous plants) live in nodules on plant roots and are the major contributors to SNF, which occurs in the root-nodules, where molecular nitrogen is reduced to ammonia by nitrogenase under anaerobic conditions. It is highly energy demanding as nitrogenase requires 16 ATP molecules for each molecule of nitrogen fixed. The entire process of nitrogen (N_2) fixation and subsequent assimilation requires 25 or more ATPs.

More than 20 genes reported to be involved in nitrogenase function have been characterized and intensively studied (Mevarech et al. 1980, Mazur and Chui 1982, Herrero and Wolk 1986, Thiel et al. 1997). Similarly, around 30 genes have been identified in legumes that are involved in internal signaling and interactions with *Rhizobium* (Glazebrook et al. 1993, Catoira et al. 2000, Santos et al. 2000, Ampe et al. 2003). The highest rate of N_2 fixation is observed in the symbiotic bacterial-root interactions rather than in the free-living soil bacteria. Estimates for the rate of N_2 fixation by non-symbiotic bacteria in the soil are in the range of 1-3 kg N ha^{-1} yr^{-1}. In contrast, certain pasture legumes can fix up to 300 kg N ha^{-1} yr^{-1} (FAO 1988).

SNF is particularly sensitive to environmental stresses, which prevent the expression of its full potential benefit for cropping systems. As a result, legume productivity can be greatly depressed when subjected to even moderate osmotic stress. Although the sensitivity of both establishment and activity of the legume-*Rhizobium* symbiosis to abiotic stress factors has long been recognized (Serraj et al. 1999), the physiological mechanisms causing inhibition of SNF are still poorly understood (see Serraj et al. in this volume). Success in decreasing the sensitivity of SNF to environmental constraints requires attention to both partners of the symbiosis, and exploitation of the new tools and methodologies of legume genomics.

Besides their deficit in nitrogen, many tropical soils, particularly in Africa, are also deficient in usable phosphorus. Some legumes improve the solubility of soil-bound phosphorus in several ways. For example, in low-input agricultural systems in the tropics pigeonpea grows and yields well in Alfisols and related soils deficient in unbound phosphorus, because it has the ability to utilize occluded Fe-P, and solubilize phosphorus for successive crops as well (Ae et al. 1990, see also Serraj et al. in this volume for more detail). However, simply growing legumes does not increase levels of nitrogen in the field since nitrogen depletion values of 12-13 kg N ha^{-1} were reported for soybean at a grain yield of 3.9-4.5 t ha^{-1} (Tanaka 1983, Tanaka et al. 1984). Thus, if much of the legume biomass is returned to the soil as green manure or pasture (e.g. *Medicago sativa*) then the amount of nitrogen provided to the soil can be as high as 85-157 kg N ha^{-1}. The potential SNF contributions of some important tropical crops are shown in Table 1:

Table 1. Potential SNF contributions of important tropical crops.

Legume species	N_2 (kg ha^{-1})	Reference
Soybean	20-260	Yamada (1974)
Cowpea	73-80	Yamada (1974)
Mung bean	61	Yamada (1974)
Groundnut	72-240	Yamada (1974)
Pigeonpea	69	Kumar Rao et al. (1983)
Chickpea	1-141	Rupela and Saxena (1987)
Alfalfa	40-350	Yamada (1974)
Clover	50-350	Yamada (1974)
Tropical legumes	100-280	Humpherys (1987)

ENHANCING SNF AND LEGUME PRODUCTIVITY

It is axiomatic that short-term impacts on enhancing biological nitrogen fixation in the tropics will come through natural resource management solutions and selection of efficient *Rhizobium* strains and legume cultivars. However, in formulating a holistic and long-term strategy towards achieving optimal levels of SNF, there are substantial opportunities for

medium-term impacts through biotech-assisted germplasm enhancement, and for long-term impacts through bioinformatic-assisted germplasm gene mining and utilization. Recent technological developments in the field of genomics have provided new tools to understand and manipulate the structure and function of entire genomes in a way never before possible (Colebatch et al. 2001). These developments in model species will fuel rapid advances in the lesser-studied tropical legumes while also providing genetic stocks and genetic populations of substantial value for more detailed studies in tropical legume crops (Journet et al. 2001, Thoquet et al. 2002, Young et al. 2003).

In committing to a holistic approach we also recognize that SNF activity is not just the consequence of a simple host-microbe interaction but also a function of interactions with a complex array of environmental conditions including the stresses of poor soil nutrient status and drought. Thus, it is critically important to move beyond single point biotechnology interventions that may result in dramatic impacts on SNF efficiency under optimum conditions. Instead we propose a systemic collaboration of disciplines from the very beginning of the innovation pipeline that will facilitate the understanding and effective manipulation of differential SNF responses under marginal production environments. Thus, our primary goal is to break the stagnation in tropical legume yields and provide technology packages that offer high and sustainable productivity under marginal cropping environments. The guiding force will be to make best use of the tools and resources already available in these crops and their model systems.

Finally, this approach would not be complete without due recognition of the critical importance of nitrogen balance, protein accumulation, nutritional status and economic value of legumes for the well being of the rural poor throughout the tropics. Here, it is essential to integrate socio-economic expertise to drive the overall context of our goals. A common feature of all legumes is the high concentration of nitrogen in the leaves, which is an important component of both green manure and fodder quality. These properties may provide many nontraditional crops with an entry point into various cropping systems for rotational purposes or to reclaim or regenerate land currently not used for crop production.

LEGUME GENOMICS AND MARKER-ASSISTED BREEDING

Marker-assisted breeding has revolutionized the improvement of temperate field crops (Toenniessen 1995, Spangenberg 2001) and will have similar impacts on plant breeding of tropical crops, particularly for traits where phenotyping is only possible late in the season, or is difficult or prohibitively expensive (Ortiz and Crouch 2003, Dwivedi et al. 2003). In the longer term,

comparative genomics and bioinformatics will allow the intensive advances in model systems to be rapidly and effectively applied to a wide range of related crops.

There are already five model legume species (*Glycine, Medicago, Lotus, Phaseolus* and *Pisum*) representing a global public and private sector genomics research investment of more than a hundred million dollars per year. Of particular interest is the large-scale sequencing of expressed genes, especially from plants that had been subjected to abiotic or biotic stress, as these will be essential entry points for driving rapid progress in the understanding and manipulation of these traits in lesser-studied crops. The vast research experience and bioinformatics databases resulting from these endeavors will form the cornerstone of the biotechnology-based approaches to enhancing SNF. Of greatest significance will be the ability to precisely and efficiently mine vast germplasm collections in order to locate rare accessions and alleles for economic applications. The seed of inbred varieties represents a powerful and sustainable means of delivering the products of advanced biotechnology.

Strategies for Legume Marker Development

Microsatellites are the marker of choice for indirect selection due to their robust, repeatable, co-dominant and high polymorphic nature. Unfortunately microsatellite markers are highly expensive to develop, thus constraining their application in lesser-studied crops. Nevertheless, even in systems where large numbers of single nucleotide polymorphism (SNP) markers are available, high throughput disease screening labs still prefer to use simple sequence repeat (SSR) markers where possible, due to the higher level of detectable polymorphism and ability to multiplex, which offers a greater level of data at lower cost

Microsatellite Enriched Libraries

Large-scale development of microsatellite markers has been funded for chickpea by BMZ (Bundesministerium für Wirtschaftliche und Entwicklung Zusammenarbeit) (Winter et al. 1999) and for pigeonpea by the UK Department for International Development (Burns et al. 2001). A traditional size fractionation approach was followed in chickpea using 4 different restriction enzymes. The genomic libraries were screened with various di- and tri-nucleotide repeat motifs, resulting in 174 microsatellite markers. In the case of pigeonpea, two enriched libraries were created using the method of Edwards et al. (1996) resulting in low recovery rate of only 10 useful microsatellite markers. Due to the substantial amount of time and cost incurred (approximately US$ 1000 per marker or more), there is considerable interest in establishing a more cost effective and labor-saving alternative in generating additional markers.

A technique known as sequence-tagged microsatellite profiling (STMP) described by Hayden and Sharp (2001) may be a more cost effective means of generating SSR markers – the technique bypasses the need for library screening.

Comparative Genomics

With the sustained interest in microsatellite markers amongst plant breeders, there has been considerable interest in the possibility of transferring SSR markers isolated from intensively studied legumes such as pea, soybean and *Medicago* to lesser-studied legumes.

A comparison of the linkage maps of *Cicer, Pisum, Lens* and *Vicia* revealed that these legumes share many common linkage groups (Gaur and Slinkard 1990; Weeden et al. 1992; Kazan et al. 1993; Simon and Muehlbauer 1997; Weeden et al. 2000). The extent of conservation of linkage arrangement may be as much as 40% of the genome (Weeden et al. 2000). The high level of conservation of linkage groups among *Cicer, Pisum, Lens* and *Vicia* suggest that these genera are very closely related. However, the use of anonymous microsatellite markers may not be the most appropriate approach for interspecific and intergeneric comparative genomics (Choumane et al. 2000).

Encouragingly, there is a 60% chance that microsatellite markers developed in pea will amplify in chickpea (although this is not definitive proof that they are amplifying a microsatellite locus in chickpea), although interestingly, less than a 20% chance in the reverse direction (Pandian et al. 2000). However, this may have more to do with the amplification conditions used in this independent report. Based on taxonomic distance, it is expected that a similar trend will be observed between soybean and pigeonpea. However, it is yet to be determined whether microsatellite loci conserved across taxonomic groups will be as polymorphic as those isolated in the crop of interest.

On this basis, ICRISAT is combining empirical lab-based approaches with bioinformatic strategies in order to develop the most efficient system for screening the vast public domain sequence databases of soybean and *Medicago* to liberate those sequences of most value for molecular breeding of chickpea and pigeonpea. Information on conserved gene sequences among these genera will also facilitate prediction of gene location in the crop of interest based on its location in other genera. This approach may be particularly useful in tagging agronomic traits that have already been intensively studied in pea, soybean and *Medicago*.

Reverse Genetics

The limited number of microsatellite markers detecting polymorphism in any given chickpea breeding population is a major constraint to molecular

breeding of agronomic traits in chickpea and many other tropical legumes. In general only a third of the microsatellite primers are polymorphic in any given mapping population, which is adequate for preliminary mapping. To find robust markers for molecular breeding of complex traits such as pest resistance, more markers are required.

For this reason, ICRISAT has pilot tested an alternative approach for the rapid development of polymorphic markers for molecular mapping and marker-assisted breeding of a particular trait of interest. In the more advanced model genomes of *Medicago* and *Arabidopsis* many expressed sequence tag (EST) libraries have been generated. In our effort to rapidly develop more markers we have tested a targeted approach for generating polymorphic EST markers specific to the trait of interest through a PCR-based cDNA subtraction technique to isolate differentially expressed sequences from roots of ICC 4958. The process has resulted in over 6600 cDNA clones, and to date a quarter of these ESTs have been sequenced, from which only 16 were found to contain microsatellite repeats. In general, plant breeders prefer SSR markers, and EST markers are rarely polymorphic in breeding populations. However, these ESTs will be valuable candidate gene markers if SNPs can be detected in the corresponding genomic region.

Comparative Legume Genomics

The collective generation and utilization of knowledge and tools across legume species will allow intractable problems in one species to be quickly resolved by cross-referencing to parallel knowledge and successes in other species. This broad-ranging knowledge and toolbox will also facilitate the effective characterization and mining of germplasm collections to identify genotypes that offer substantially higher SNF efficiencies under marginal cropping environments (including drought and salinity stress and low soil phosphorus).

Comparative mapping was first carried out extensively in the cereal crops (Gale and Devos 1998). Thus, a large number of extensive comparative genomics studies have already been completed amongst cereal species and much of this information is presented at the Gramene portal (Ware et al. 2002). Despite large differences in DNA content and chromosome number, the grass genomes maintain a high level of conserved macro-synteny and a moderate to high level of micro-synteny. This has led to a diverse array of initiatives based on extrapolating and cross-referencing to rice as the model hub for the grass species. More recently rapid and intensive progress has been made in comparative mapping amongst legume species (Weeden et al. 2002, Kulikova 2001).

Over the past decade, plant breeders and geneticists have made progress in understanding genome organization, diversity and evolutionary

relationships in several major crop groups (e.g. grasses, solanaceous and legumes crops). Many genes are highly conserved across plant families. Comparative genetics provides the potential for trait exploitation from species where genetic control is well understood and for which there are many molecular markers, to a species that has a more limited amount of information. Genetic and physical maps of rice can be used as reference points for the larger and more complex genomes of the major and minor cereal crops (Wilson et al. 1999). In addition breeding efforts and molecular analysis of barley, wheat and maize have had direct applications in rice improvement (SGRP 1999). Comparative genetics will facilitate the identification of desirable alleles in genepools close to the target crops, which can be introgressed in breeding programs through marker-assisted introgression techniques.

For lesser-studied crops in developing countries this approach offers a considerable logistical advantage. Researchers working on these crops can access information developed in advanced labs and apply this directly in their crop of interest using adapted robust technologies. In this way scientists in developing countries can carry out highly innovative and relevant work in situ. For the development, refinement and application of effective molecular breeding systems, this proximity to the target environment and breeding program is necessary.

Comparisons between the legumes using genomic tools may show why certain legumes are better at N_2 fixation than others. Accessions highly efficient in SNF appear to exist in all major crop species. For example, although common bean is often considered as a poor N_2-fixing legume, more than 20 years of research at CIAT has identified high N_2-fixing lines of bean (Bliss 1993). This suggests that immediate impact can be achieved through the development of effective selection tools to introgress the component traits into the primary gene pool of a crop. This is a situation similar to drought tolerance breeding, where a complex and difficult-to-select trait can be sequentially lost from breeding populations due to the absence of positive selection pressure.

There is no doubt that modern genomics can provide precise and cost effective solutions to this problem. Analysis of segregating populations has shown that quantitative traits such as N_2 fixation capacity or drought tolerance are controlled by a limited number of genetic loci, so called quantitative trait loci (QTL). These results offer the opportunity to select genes for SNF capacity in legumes using molecular markers. Complementing the QTL analysis is the search presently being conducted by various laboratories for the genes involved in efficient nitrogen fixation. This is termed the "candidate gene approach" as genes expressed at the right physiological time point, in the right tissue and at the right genomic position are likely to be the candidates that determine QTLs that are found in genetic mapping studies.

Diagnostics

The recent development of DNA chip technology combined with PCR diagnostics has enabled the identification of human pathogens in a matter of hours as opposed to current diagnostic techniques requiring sequential tests for many different organisms, which takes many days. These technologies will prove highly powerful for large-scale detection of symbiotic microbes, pathogens and for marker-assisted selection of complex agronomic traits in plant breeding.

Functional Genomics

With the advent of microarrays it is now possible to survey the genes expressed by different genotypes at various developmental stages and in response to biotic or abiotic stresses. This will be an important tool for the dissection of complex traits such as drought tolerance and SNF. Functional genomics will facilitate screening of germplasm for diversity in gene expression and allow a functional-based prioritizing amongst the vast array of QTL identified during precise mapping of complex traits. A first pilot chip containing 150 stress-responsive cDNAs from legumes is already available and has been useful for analyzing stress-responses in chickpea (Winter et al., this volume). Improved chips built on knowledge gained with such pilot developments will speed up the screening of legume germplasm provided they can be produced and utilized at reasonable costs.

Serial analysis of gene expression (SAGE)(Velculescu 1995) is a process for analyzing gene expression patterns in any eucaryotic organism. The major advantage of SAGE over traditional microarray technology is that the expression of unknown genes can be analyzed. In the case of rice, ten unidentified genes were expressed in rice seedlings at levels differing significantly between samples grown under anaerobic and aerobic conditions (Matsumura et al. 1999). In addition, no special device is required for SAGE other than a DNA sequencer. For meaningful application of SAGE there has to be a pre-existing EST database available, for example, the soybean mature root nodule library (of over 6000 EST) will be a powerful resource.

Some legumes such as cowpea and many forage plants are very efficient N_2-fixers, producing up to 80% of their nitrogen needs. In contrast, some legumes such as common beans are relatively poor N_2-fixers. This may reflect recent selection under high input conditions. A consensus legume approach to SNF will facilitate better understanding and manipulation of SNF efficiency in all legume crops.

Current Understanding of Biological Nitrogen Fixation

Ambitious predictions several decades ago regarding the likely impact of SNF on world agriculture led to a diverse intensification in this research area. Although of less impact than expected, our understanding of the microbiology, physiology, biochemistry and molecular biology of legume symbiosis has tremendously advanced. A great deal is now understood about the molecular events involved in recognition between rhizobial genes known to control host specificity and nodulation that have been classified into structural *nod* genes and regulatory *nod* genes (Geurts et al. 1997, Hirsch and Kapulnik 1998, Cárdenas et al. 2000, Stoutgaard 2001, Amor et al. 2003, Loh and Stacey 2003, Shaw and Long 2003). Plant root exudates (particularly isoflavonoids) stimulate activity of the structural nod genes, thus driving nodulation. There is a specificity system between these isoflavonoids and the rhizobial nod genes. The flavonoids enter the bacterial cells and bind to the NodD protein which acts as a transcriptional activator for other nod genes and the 'Nod factor', which then triggers the host response leading to *Rhizobium* infection and ultimately nodule formation. Another important component of the root symbiosis system is fungal symbionts, so called mycorrhiza, which can significantly enhance phosphate uptake. Mycorrhiza and *Rhizobium* symbioses are fundamentally different processes controlled by different gene families of the plant. Yet, a number of loci have been shown to be important for both processes in *Lotus*, *Medicago* and pea. These loci are probably involved in root hair response and eventually encode signaling factors (Cárdenas et al. 2000).

Large-scale sequencing, particularly of ESTs, is a fundamental activity in all the model species. Currently, the largest number of ESTs is in soybean (over 250,000) but progress in *Medicago* and *Lotus* will quickly lead to similar numbers in these species including libraries derived from symbiotic or biotically and abiotically stressed tissues. These rapidly expanding databases are being cross-referenced with each other and compared to the distantly related model *Arabidopsis* for establishing conserved genes. Comparative sequence analysis amongst legumes compared with *Arabidopsis* will allow legume-specific genes to be differentiated from more general housekeeping genes.

Fewer genomic resources have been developed in the food crop legumes than in the models and there is an urgent need to develop cDNA libraries for nodule-specific and abiotic stress tolerance genes from common bean, pigeonpea, chickpea and others. These libraries will facilitate identification of EST sequences homologous to genes that have been characterized in model plants as *Medicago* or *Lotus* and advanced crops such as soybean.

An important advantage of the model legume species are the hundreds of well-characterized mutant stocks that can be used to precisely study trait

components influencing SNF. There are mutants with super-, hyper-, early or no nodulation, root hair deformation and mutants with different host-strain interactions that constrain or completely inhibit bacteroid differentiation and/or nitrogen fixation. Large-scale insertional mutagenesis programs are also underway in many model species that will lead to vast arrays of genetic stocks and isolated genes.

Mutant loci are increasingly being physically mapped to specific bacterial artificial chromosomes (BACs), but considering the large number of loci involved in N_2 fixation it may not be feasible to adopt a transgenic approach to this problem. However, transformation studies are becoming increasingly frequent in *Medicago* and *Lotus*. Host plants will be transformed with cDNAs encoding the complete open reading frames of apoplastic proteins. Some cloning vectors, for example PCLD04541, allow direct *Agrobacterium*-mediated transformation of BAC clones into plants. The BACs will be engineered with appropriate reporter genes to allow subsequent cytological and biochemical study. A second range of probes will be obtained by developing antisera to conserved peptide motifs. These technological developments provide a unique opportunity to exploit the progress in the models for the improvement of lesser-studied crops and thereby quickly achieve impact in farmers' fields.

Nodulation and N_2 fixation are highly complex, involving a large number of genes, including those influencing plant growth and development. Functional genomics studies in model species are facilitating the study of gene families and the entire complement of genes contributing to a given process (Colebatch et al. 2001, Ampe et al. 2003). This shift away from the analysis of single genes will allow molecular biologists to effectively contribute to holistic studies of complex phenomena. Through a combination of comparative genomics, bioinformatics and functional genomics it should now be possible to identify universal loci involved in plant signaling, nodule development and N_2 fixation (see Fig. 2). Already several genes involved in *Rhizobium* symbiosis have been mapped to clusters in the pea genome and homologous clusters identified in *Medicago*. The goal of this research is now to determine which of these genes are most important for the symbiotic process.

Environmental Interactions With Nitrogen Fixation

The interaction between N_2 fixation and abiotic stress tolerance is an important area of research. Among the prominent abiotic stresses faced by legumes in the tropics are drought, salinity, acid soils (aluminium and manganese toxicity) and phosphorus deficiency. All of these and especially low phosphorus adversely affect SNF, delivering a double blow to crop productivity. Low N_2 fixation associated with low phosphorus content of their soils often affects small farmers lacking access to commercial fertilizers.

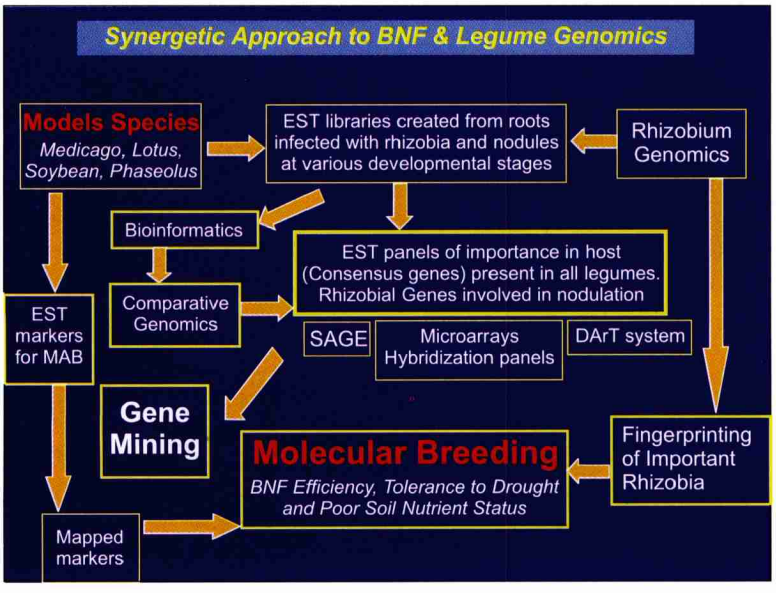
Synergetic Approach to BNF & Legume Genomics
Models Species
Medicago, Lotus, Soybean, Phaseolus
EST libraries created from roots infected with rhizobia and nodules at various developmental stages
Rhizobium Genomics
Bioinformatics
EST panels of importance in host (Consensus genes) present in all legumes. Rhizobial Genes involved in nodulation
EST markers for MAB
Comparative Genomics
SAGE
Microarrays Hybridization panels
DArT system
Gene Mining
Molecular Breeding
BNF Efficiency, Tolerance to Drought and Poor Soil Nutrient Status
Fingerprinting of Important Rhizobia
Mapped markers

Figure 2. Holistic integration of genomics and bioinformatics.

A long-standing collaboration between CIAT scientists and INRA has resulted in the identification of lines of common beans, such as BAT477, with good N_2-fixing potential despite relatively low phosphorus supply (Vadez et al. 1999). Why these particular genotypes are more efficient is still not fully understood, although progress has been made in understanding their physiology (Vadez and Drevon 2001). Some elite N_2-fixing lines have been used as parents to develop segregating populations for the study of phosphorus use efficiency and N_2 fixation ability under replicated field conditions.

Nitrogen fixation under phosphorus deficiency affects several physiological characteristics of the nodule such as N_2-dependent growth, nodule respiration and control of oxygen diffusion. The nodular cortex is particularly important for the effective function of the nodule by controlling oxygen permeability and nutrient transport, processes that are negatively influenced by nutrient deficiency, salinity, or drought stress (Serraj 2002). The involvement of aquaporin and carbonic anhydrase genes in controlling nodule permeability has also been implicated by *in situ* hybridization studies (Serraj et al. 1998, de la Pena et al. 1997). Differential display has been used to discover other candidate genes that are expressed specifically in the peripheral nodule cortex tissues (Gherbi et al. 2000). New candidate genes are being analyzed by in situ hybridization using dissected nodules or roots in order to localize and quantify gene expression. It is hoped that this will

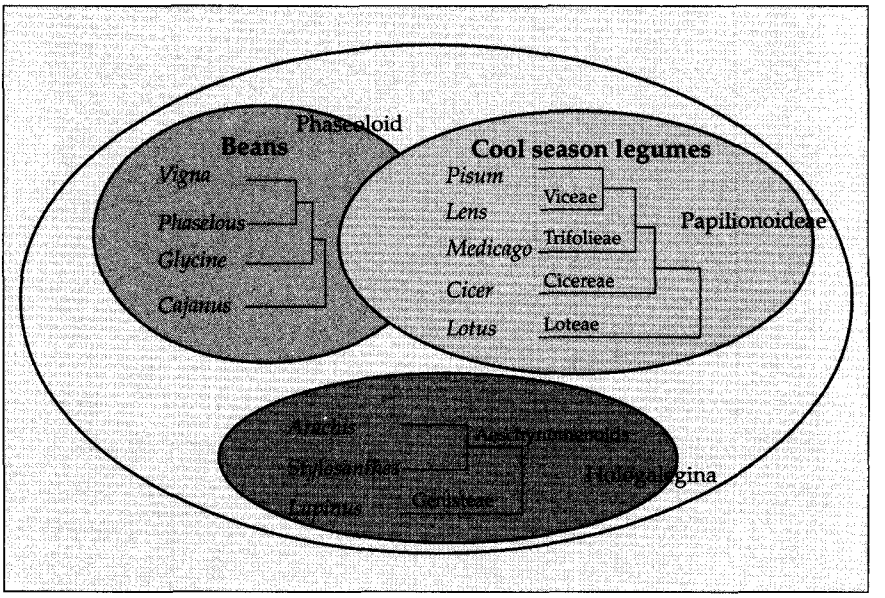

Figure 3. Diagrammatic representation of legume taxonomy for major crops, model species and lesser-studied crops (phylogenetic distances not to scale).

lead to progress in understanding mechanisms associated with tolerance to abiotic stress, and allow the identification of genes conferring tolerance. Mechanisms that are of special interest include the ability to redistribute phosphorus, to solubilize internal and external organic-P sources, or to modify nodule oxygen permeability through nitrogenase-linked respiration and proton efflux.

Manipulating Rhizobia

Through conventional breeding of some legume crops at least one nodulation inhibitor gene has arisen that does not have a corresponding nodulation gene in *Rhizobium*. Thus, rhizobial strains that have been genetically engineered to contain the desired root nodulation genes for the target legume crop are expected to have a competitive advantage over indigenous or undesired rhizobia.

Similarly, mutant rhizobia strains have been selected with an inactive 'rai I gene' that leads to increased nodulation in bean (Wisniewski-Dye et al. 2002). Based on this it is proposed that other specific strains can be mutated in a similar way. This suggests that rhizobial strains can be created that both enhance the symbiosis and maintain competitive advantage in the soil environment.

CONCLUSIONS

A Framework for Linking Research on Model legumes With Practical Outputs for Related Crops

Legume species can be clustered into three broad groups (Doyle et al. 1997, Kajita et al. 2001), showing that most of the economically important legumes in the tropics (except groundnut) are taxonomically close to at least one model species or major crop.

Based on these close phytogenetic relationships, it will be possible to extrapolate the advances in genetics from at least one and perhaps all model species on the rest of related legume species. On this basis, the international legume genomics initiative is striving to define a 'consensus legume' through comparative genomic analysis of more than 500 PCR - based markers screened across this group of species and anchored to physical maps of *Phaseolus* and *Medicago.*

The combination of rapid advances in the model species with this wide-ranging comparative mapping initiative will provide an extremely powerful platform for trait-oriented research teams to focus on pest and disease resistance, SNF, drought and poor soil nutrient status stress. On this basis it will be possible to identify gene-based markers for QTL contributing to each component trait influencing SNF in various legume species. Next, bioinformatics approaches must be used to identify consensus genes critical to the SNF process that are conserved across legume species. These marker sequences can be consolidated on microarray hybridization panels and used for gene mining of germplasm collections in a parallel way to that proposed using the DarT system (Jaccoud et al. 2001). In the absence of specific gene sequence information, lesser-studied crops can still participate in this approach to a certain level through QTL mapping of component traits followed by the use of these markers on microarrays (Jain et al. 2001).

Developing more precise means of screening large germplasm collections for legume SNF stress tolerance is a critical issue. Typically, entire germplasm collections of 10,000 to 50,000 accessions per crop, available at CGIAR centers, have been evaluated under field conditions for various traits. However, very often sources of pest and disease resistance have not been identified through this process. This has recently been highlighted following the development of core collections (van Hintum 1999, 2000). Here just a few hundred accessions are selected to represent the whole collection. When entomologists and pathologists intensively studied these smaller collections, a large number of sources of resistance were generally identified (Greene 2001, Huamán 1999, Ortiz 2002). The same methodology could also be applied to screen legume germplasm for high SNF potential and stress resistance.

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4

Improvement of Legume Productivity and Role of Symbiotic Nitrogen Fixation in Cropping Systems: Overcoming the Physiological and Agronomic Limitations

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ABSTRACT

Nitrogen-fixing legumes can meet most of their N-needs through symbiotic nitrogen fixation (SNF). However, in most cases, inclusion of a legume in a cropping system does not ensure the attainment of such levels of SNF in the field. Several environmental factors including drought, temperature and soil nutrient status dramatically affect the process at molecular/functional level and thus play a part in determining the actual amount of nitrogen fixed by a given legume in the field. This chapter reviews the status of SNF in response to most significant environmental constraints, and focuses on specific cases of harnessing SNF by improving its tolerance to stress factors with the aim of enhancing system productivity. Several examples are discussed, including the selection of legume crops tolerant to drought and salinity and/or allowing high biomass production and solubilization of phosphorus, identifying high nitrogen-fixing and nitrate-N tolerant genotypes and their inclusion in relevant cropping systems, and changes in agronomical management practices for better integration of legumes in cereal cropping systems. Finally, a general framework is discussed for agro-physiological contributions that can help overcome SNF limitation by environmental constraints. The on-farm application of these knowledge-based SNF technologies will strengthen the role of N_2-fixing legumes in cropping systems.

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INTRODUCTION

Symbiotic nitrogen fixation by legumes plays an important role in sustaining crop productivity and maintaining fertility of marginal lands and in smallholder systems of the semi-arid tropics. It is anticipated that the importance of legumes and SNF will continue to expand with the increasing development of sustainable agricultural practices and growing concern and awareness about the environment. The first step toward maximizing SNF technologies is to increase the land area under legumes and enhance their grain and fodder yields through overcoming environmental limitations of SNF and legume productivity.

Substantial qualitative information is available on the net benefits of SNF and its residual effects on grain, herbaceous, and tree legumes. However, SNF by legumes is particularly sensitive to various environmental stresses such as drought, waterlogging, soil salinity or acidity, temperature, insect-pests, diseases, and low phosphorus (P) and other nutrient limitations. Consequently, legume productivity can be greatly depressed if subjected to these environmental constraints. For instance, the sensitivity to drought and salt stress of both establishment and activity of the legume-*Rhizobium* symbiosis has long been recognized (Wilson 1931, Bernstein and Ogata 1966). Although drought and salinity effects on N_2 fixation have been extensively studied in several legume species, the physiological mechanisms involved in the inhibition are still poorly understood. The N_2-fixing legume plants usually require more P than plants dependent on mineral N fertilizer. Nodule establishment and function are important sinks for P, and nodules usually have the highest P content in the plant. Therefore, P deficiency conditions result in reduced SNF potential and P fertilization will usually result in enhanced nodule number and mass, as well as greater N_2 fixation activity per plant.

This paper is one of four background documents that analyze the various component approaches to SNF, including legume genomics (Crouch et al., op cit), participatory approaches (Twomlow et al., op cit) and socioeconomic and policy issues (Shiferaw et al., op cit). The document focuses on the agro-physiological constraints that limit SNF potential, and the agro-physio-genetic resilient traits associated with legume genetic tolerance as well as management options to deal with drought, soil salinity and acidity, nutritional stress, and temperature. Information provided about some of the candidate mechanisms will strengthen the knowledge base for initiating genetic manipulation and eventual gene transfer to enhance the productivity of legumes in the semi-arid environments. Moreover, such knowledge will facilitate development of appropriate management options for harnessing benefits of increased SNF contributions in these systems.

ENVIRONMENTAL STRESS AFFECTING SNF PROCESSES

Most stress factors influence all physiological processes in plants as the stress develops (Table 1). They influence all aspects of nodulation and symbiotic N_2 fixation, in some cases reducing rhizobial survival and diversity in soil, in others essentially affecting nodulation and nitrogenase activity. It is often difficult to isolate the effects of the stress factors on the success of inoculation from their effects on symbiosis functioning and N_2 fixation. The most important stresses include abiotic factors such as drought and salinity, waterlogging, temperature, soil acidity, and inadequate mineral nutrition (Table 1), and biotic factors such as insect-pests and diseases. A critical question with regard to N_2 fixation is whether the stresses first affect other physiological processes, which then influence N_2 fixation, or whether the stress initially and directly affects N_2 fixation mechanisms. Physiological understanding of the most stress-sensitive steps is also essential for establishing strategies for crop improvement and adequate management practices to optimize legume N_2 fixation and increase its role in cropping systems. For instance, N_2 fixation has been found more sensitive to soil dehydration than leaf gas exchange (Sinclair et al. 1987, Djekoun and Planchon 1991), nitrate assimilation (Purcell and King 1996) and dry matter accumulation (Sinclair et al. 1987, Wery et al. 1994). Similarly, several studies have shown that N_2 fixation was more sensitive to salt stress than plant growth (Delgado et al. 1994, Serraj and Drevon 1998).

Rupela and Rao (1987) showed that legume-*Rhizobium* symbiosis is particularly sensitive to drought, salinity and extremes of temperature in chickpea and pigeonpea plants, much more so than rhizobia growing alone. All three stress factors were found to impair the development of root hairs and the site of entry of rhizobia into the host, resulting in poor or no nodulation. Salinity and high temperature affected nodulated plants more than they did for N-fertilized plants.

The existence of genetic variability in tolerance to most environmental stress factors has been shown in both legume host plants and their respective rhizobial strains (see review by Hungria and Vargas 2000). This suggests the possibility of overcoming, at least partly, the environmental constraints limiting legume SNF potential. Success in decreasing the sensitivity of legumes to environmental stress would be achieved by focusing on both partners of the symbiosis, although it is generally agreed that *Rhizobium* strains are relatively more tolerant than the corresponding host plants (e.g. in the case of salt tolerance, Singleton et al. 1982). Similarly, compared to host plants, rhizobial strains are quite resistant to soil desiccation, and can survive in water films surrounding soil particles (Williams and de Mallorca 1984).

The current challenges are to understand the mechanisms responsible for stress sensitivity at the level of the whole plant and to improve the tolerance

Table 1. Effects of major environmental constraints on symbiotic nitrogen fixation. Number of papers found in databases and effects of various constraints on legume-*Rhizobium* establishment and functioning processes.

Constraint	Number of References (Agricola, 1979-2003 and CAB Abstracts, 1973-2003)[a]	Processes affected			Key references and reviews
		Rhizobium survival	Nodule formation & growth	N_2 Fixation	
Light	265	–	* (b)	**	Hirsch and McFall-Ngai 2000, Swaraj et al. 2001, Walsh 1995.
Temperature	328				Rennie and Kemp (1982),
Low		**	***	**	Day et al. (1978), Piha and Munns (1987b),
High		**	***	**	Roughley et al. (1995), Rupela and Rao (1987)
Carbon dioxide	66	*	***	**	Hardy and Havelka 1976, Hebeisen et al. 1997, Cabrerizo et al. 2001, Serraj et al. 1998b
Oxygen	221	–	**	***	Minchin 1997, Walsh et al. 1989, Walsh 1995
Drought (or water stress)	341	*	**	***	Serraj et al. 1999b, Sinclair et al. 1987, Williams and de Mallorca 1984
Salinity	149	*	**	***	Rupela and Rao 1987, Serraj et al. 1998a, Singleton et al. 1982, vanHoorn et al. 2001
Nitrate	655	*	***	***	Herridge et al. 1994, Streeter 1988, Walsh 1989
Soil acidity	498	**	**	**	Cooper 1988, Glenn et al. 1988, Hungria and Vargas 2000
Low phosphorus	136	–	**	***	Cassman et al. 1981, Israel and Rufty 1988, Vance 2001, Sinclair and Vadez 2002
Micronutrients	20	–	**	**	O'Hara et al. 1993, Ali and Mishra 2000, Giller 2001

(a)Search with key words 'legume' and 'nitrogen fixation', combined with the various constraints.
(b)The (*) symbols indicate relative level of process sensitivity to the constraint.

of N_2 fixation to abiotic and biotic stress factors. The need to assess genetic diversity of legumes in terms of SNF potential, in order to screen and utilize available legume germplasm for efficient SNF is an important first step. This may also offer a critical resource in physiological investigations and plant breeding efforts targeted at increasing SNF in relevant cropping systems.

Drought Stress

Legume productivity in the semi-arid tropics (SAT) is largely limited by low soil moisture availability in addition to nutrient deficiencies. The relatively high sensitivity of nitrogen and biomass accumulation to soil dehydration under field conditions was demonstrated for soybean grown on a soil with virtually no mineral N reserve (Sinclair et al. 1987, Serraj and Sinclair 1997). With essentially all N uptake resulting from N_2 fixation, a comparison of biomass accumulation and N accumulation rates offered an index of the relative sensitivity to water-deficit conditions under which the plants were grown. Sinclair et al. (1987) concluded from their study on soybean that N_2 fixation was more sensitive to drought than was carbon assimilation. In a similar study on 24 soybean lines, Serraj and Sinclair (1997) found that in almost all the soybean cultivars tested N accumulation was more sensitive to soil dehydration than was biomass accumulation. This conclusion from field studies was supported by detailed observations in glasshouse studies, which showed that the effects of soil dehydration on N_2 fixation, as measured by an acetylene reduction assay, occurred at much higher soil water contents than the effects on C accumulation (Serraj and Sinclair 1997).

The fact that N_2 fixation is more sensitive to decreasing soil water content relative to leaf gas exchange is an important constraint on N accumulation and the yield potential of legumes subjected to soil drying (Sinclair et al. 1987, Wery et al. 1994). For cool-season food legumes such as chickpea, Beck et al. (1991) concluded that even if the drought stress effects on N_2 fixation do not always directly affect grain yield, drought may result in a significant decrease in the total N balance. Loss of N_2 fixation under water deficits would then reduce the advantage of using legume crops in rotations, for green manuring and soil fertility improvement.

Although it is recognized that drought-tolerant varieties have evolved with different traits, the traits most often specified are those of roots. A deeper root system with enhanced water uptake capacity is considered synonymous with drought avoidance in many crops (Gregory et al. 1994). Thus, legumes with deep root systems are preferentially grown in climates with limited rainfall, where they can withstand prolonged periods of drought.

Substantial efforts have been devoted to selecting and breeding legumes tolerant to drought, but with very little success because of the complexity of

the genes controlling drought. More important, the simulated drought environment in which screening is conducted is often not well defined and therefore not reproducible. Accurate field phenotyping of mapping populations for traits associated with drought tolerance requires extra efforts in conceptualization, design, and management of phenotyping programs, to maximize the chances of identifying quantitative trait loci (QTL) that will be useful in future improvement of tolerance in the target crop and the target environments (Bidinger 2001). Establishing screening conditions representative of the larger environment is difficult, involving major trade-offs between providing representative day length, vapor pressure and temperature conditions.

Chickpea

The vast majority of chickpea produced worldwide is grown by resource-poor farmers under rainfed conditions, usually planted after the main rainy season and grown on declining soil moisture. Terminal drought tolerance is, therefore, a primary constraint to chickpea productivity. Significant progress has been made in developing improved chickpea varieties of short duration that mature in 70-90 days in mild winter chickpea growing conditions, able to escape terminal drought (Kumar et al. 1996). Even extra-short duration (ESD) chickpea varieties, termed *super-early* have now been developed (Kumar and van Rheenen 2000). Development of these new varieties has expanded the options of including chickpea as a crop in many prevailing and evolving new production systems, such as the rice fallows of South Asia (Musa et al. 2001). This work needs, however, to be extended to include other key legume crops, with a specific focus on SNF, to overcome the soil fertility and environmental constraints.

Large and deep root systems have been characterized as important drought avoidance traits, useful in greater extraction of available soil moisture, and have been widely used for the genetic enhancement of chickpea under terminal drought (ICRISAT 1992). The routine application of molecular markers, combined with the use of adequate genetic populations offers a paradigm shift in the ability to study and manipulate root traits. The chickpea line ICC 4958 has the multiple drought avoidance traits of large root size, a rapid rate of root development and extraction of water, and a rapid seed development rate related to its large seed size. Recombinant inbred lines (RIL) of a chickpea cross (ICC 4958 x Annigeri) have also been phenotyped for root traits. Identification of QTLs for the large root system of ICC 4958 to develop a marker-assisted selection technique is currently in progress (Krishnamurthy et al. 2003, Kashiwagi et al. 2003, Chandra et al. 2003, in preparation).

As an amide producer, chickpea has been found relatively tolerant to drought in terms of N_2 fixation response, compared to ureide producers

(Sinclair and Serraj 1995). However, more work is needed to investigate the genetic variability of nodulation and SNF response to water deficits.

Pigeonpea

Important putative drought tolerance traits in pigeonpea include early vigor, leaf area maintenance, root and shoot growth rate and plasticity in development (Johansen 2001). Early growth vigor is an important factor in drought resistance as it permits establishment of a root system that is very effective in extracting water during later drought periods. This is considered the main reason for the better growth and yield of pigeonpea hybrids such as ICPH 8 and ICPH 9, compared to varieties from which they are derived, under both drought and well-watered conditions.

There are considerable differences in early growth vigor of different pigeonpea varieties (Johansen 2001). Early-maturing genotypes generally show more vigor than later-maturing ones, with hybrids showing most vigor, but there are exploitable differences in this trait within maturity groups. The recently developed extra-short duration (ESD) genotypes mature in less than 110 days, with yield potentials comparable to short- and medium-duration cultivars when grown under adequate moisture supply (Chauhan et al. 1992). However, ESD genotypes are poorly adapted to rainfed conditions because their shallow rooting behavior makes them susceptible to drought stress, particularly during flowering and pod filling, resulting in severe yield losses (Nam et al. 2001).

Despite the demonstrated ability of pigeonpea to grow in N-deficient soils without inputs of N-fertilizer (Kumar Rao et al. 1983), the quantification of the amounts of N_2 fixed has proved difficult (Peoples et al. 1989). However, the xylem ureide assay has been successfully used for the quantification of SNF capacity in pigeonpea (Peoples et al. 1989). The drought response of SNF in pigeonpea has previously received little investigation. However, there is indication that as a ureide producer, pigeonpea is likely to be essentially drought sensitive (Serraj et al., unpublished data). The genetic variability of nodulation and SNF under drought in the various maturity groups of pigeonpea needs to be further investigated.

Groundnut

ICRISAT has adopted a holistic approach in screening and selecting groundnut varieties with super performance at the two most critical stages of drought (mid- and end-season). Several such lines are now available for use in breeding programs. The physiological basis of genotypic response to drought in groundnut was identified as involving Harvest Index (HI), total amount of water transpired (T) and transpiration efficiency (TE). Genotypes derived from parental lines selected in field drought screening at ICRISAT showed superior yield performances because of higher TE and HI, while for

other cultivars, the dominant contribution to the yield was T and/or HI. The T and TE were estimated indirectly from SPAD-chlorophyll meter readings, specific leaf area (SLA) and specific leaf nitrogen (SLN).

More recently, dry-down experiments were carried out under controlled environment for the analysis of TE and stomatal regulation under water deficit, in relation to nodulation and plant N status. Evaluation of genetic variability in plant water use and leaf gas exchange responses to soil drying has been carried out in RILs selected for high water use efficiency (comparison of lines with high and low TE from previous selection experiments). The data confirmed the genotypic variation observed previously in the field in total amount of water transpired and TE. These data have also shown that TE in groundnut leaves is correlated with SLA, nodulation and N status (Serraj et al., unpublished). This currently ongoing work aims to confirm the link between TE and nodulation under drought and for the development of genetic linkage mapping. This will facilitate the characterization of QTLs and offer practical means for manipulating the underlying traits for water use efficiency in groundnut breeding programs.

Other Legume Species

The variability of N_2 fixation sensitivity to drought has been analyzed with several grain legumes including soybean, cowpea, black gram, chickpea, common bean, faba bean, lupine, pea, and peanut (Sinclair and Serraj 1995). The results obtained from soybean and cowpea showed that the sensitivity of acetylene reduction activity (ARA) in these species to soil drying was greater than transpiration in nearly all cases. Surprisingly, all other grain legumes showed that ARA was less sensitive to water deficits than was transpiration during the water-deficit period (Sinclair and Serraj 1995). The drought tolerance trait was associated with the biochemical form of N exported by the nodules, with ureide transporters being more sensitive than amide producers.

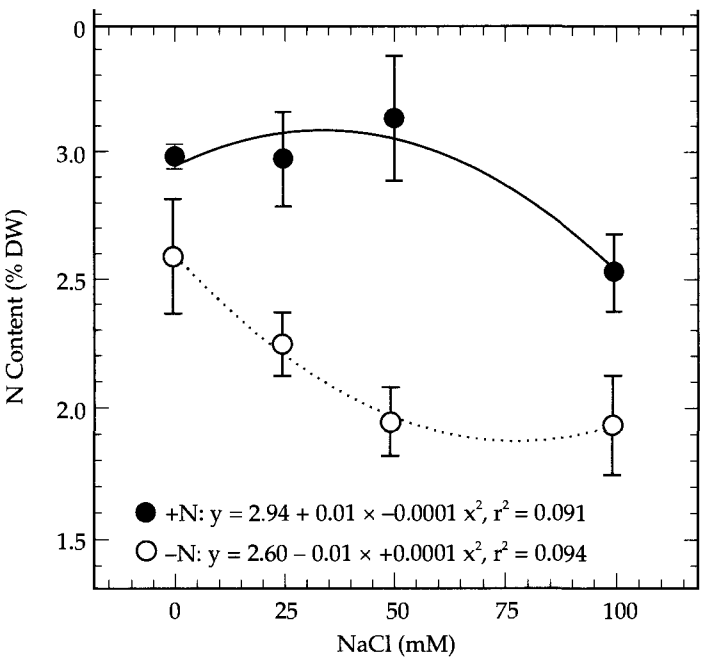
Serraj et al. (1999a) investigated the inhibition of N_2 fixation in soybean due to water deficits and showed that ureides (allantoin and allantoic acid) were involved in the sensitivity to drought. Consistent with this observation, variability in N_2 fixation sensitivity among legume species and cultivars to water deficit has been associated with the amount of ureides that they accumulate (Serraj et al. 1999a). Further evidence of the importance of ureides in the sensitivity of N_2 fixation to water deficit is the substantial increase observed in ureide concentrations in soybean shoots and nodules upon soil drying (Purcell et al. 1998, Serraj et al. 1999b).

Other promising and under-utilized legume species include 'arid legumes' such as mothbean (*Vigna aconitifolia*), tepary bean (*Phaseolus acutifolius*), clusterbean (*Psophocarpus tetragonolobus*), horsegram (*Dolichus biflorus* L.), Bambara groundnut (*Vigna subterranea*) and cowpea (*Vigna unguiculata* L.),

which are all well adapted to arid and semi-arid areas under very limited water resource conditions. However, despite their potential importance in sustainable agriculture in drylands, few studies have focused on the factors limiting the production and wider use of these legume species in the arid and semi-arid zones.

Salinity

It was reported that rhizobia can generally tolerate a higher level of salinity than the host legume (Singleton et al. 1982). Fast-growing rhizobial strains are more salt-tolerant than slow growing ones. Subbarao et al. (1990) observed significant differences among pigeonpea *Rhizobium* strains in their ability to nodulate and fix nitrogen with a pigeonpea genotype under saline conditions, and further observed that nodule initiation was the most salt-susceptible aspect of pigeonpea growth. Wild pigeonpea species (*Cajanus platycarpus* and *C. albicans*) have been reported to tolerate salinity up to 12 dS m^{-1}, compared to 6 dS m^{-1} for cultivated species (ICPL 227). Mechanisms for salinity tolerance in pigeonpea involve exclusion of Na^+ and Cl^- ions from the shoot, and the maintenance of high K levels.



Source: Data of Serraj and Drevon 1998.

Figure 1. Effect of NaCl concentration on alfalfa (*Medicago sativa* L.) N content in presence (+N) or absence (-N) of 3 mM nitrate.

The effects of salinity on biomass and N accumulation in alfalfa were compared in plants fed with nitrate (NO_3) or dependent on N_2 fixation (Serraj and Drevon 1998). NaCl inhibited nitrogen accumulation in both NO_3-fed plants and N_2-fixing plants, which was seen as a decrease in N concentration (%N in plant biomass). The decrease was larger for N_2-fixing plants than for NO_3-fed plants (Fig. 1). The %N in NO_3-fed plants was not affected by low concentrations of NaCl (up to 50 mM), whereas N_2-fixing plants showed a significant decrease in %N with increasing levels of NaCl. Below 50 mM NaCl, %N was about 100% and 75% of that in the control plants for NO_3-fed plants and N_2-fixing plants, respectively. The absence of a significant effect of low NaCl concentrations on %N in NO_3-fed plants supports previous reports showing inhibitory effects of NaCl on growth without any decrease in %N (Pessarakli and Zhou 1990, Cordovilla et al. 1995). This contrasts with the relatively large effect of NaCl on %N content of N_2-fixing plants (Serraj and Drevon 1998). Therefore, N accumulation appears to be more salt sensitive in N_2-fixing plants than in NO_3-fed plants, demonstrating the higher sensitivity to salt stress in N_2-fixing plants.

A recent study of four grain legumes including broadbean, chickpea, lentil and soybean confirmed the effects of soil salinity on crop yield, total nitrogen uptake and N_2 fixation (van Hoorn et al. 2001). The existence of inter- and intraspecific variability in the sensitivity of N_2 fixation to salinity has also been reported in legumes (Serraj et al. 1998a, 2001). The level of N_2 fixation sensitivity to salt stress was associated with the level of salt accumulation in the nodules. Exposure to NaCl increased the Na^+ and Cl^- content of all plant tissues and cultivars, although the content was higher in nodules than in shoot tissues. Nodules in common bean accumulated higher NaCl levels compared to those of soybean and alfalfa (Serraj et al. 1998a), which confirmed previous evidence that salt tolerance in mesophytes was correlated with ion exclusion (Greenway and Munns 1980). Furthermore, legume species and cultivars differ in ion distribution and especially the ratio of Na/K within plant organs (Ortiz et al. 1994, Cordovilla et al. 1995). However, little information is available on the effect of salt on ion distribution in legume nodules.

Overall, the existence of genetic variability among legume species and cultivars in the sensitivity of N_2 fixation to salt may prove useful in further elucidating the mechanism of NaCl inhibition of SNF and in selection of optimal *Rhizobium*-legume symbioses for agricultural production in saline soils.

Availability of Nutrients

In the less fertile rainfall-deficient regions of the SAT, no improved cultivar has a reasonable chance of achieving substantial and sustainable yield in

the farmer's field unless the critical constraints of soil fertility are addressed. Whereas a lot of effort has gone into breeding for disease-, pest- and drought-resistant crops, little attention has been devoted to identifying and exploiting physio-genetic systems that increase the uptake and utilization efficiencies of legume crops.

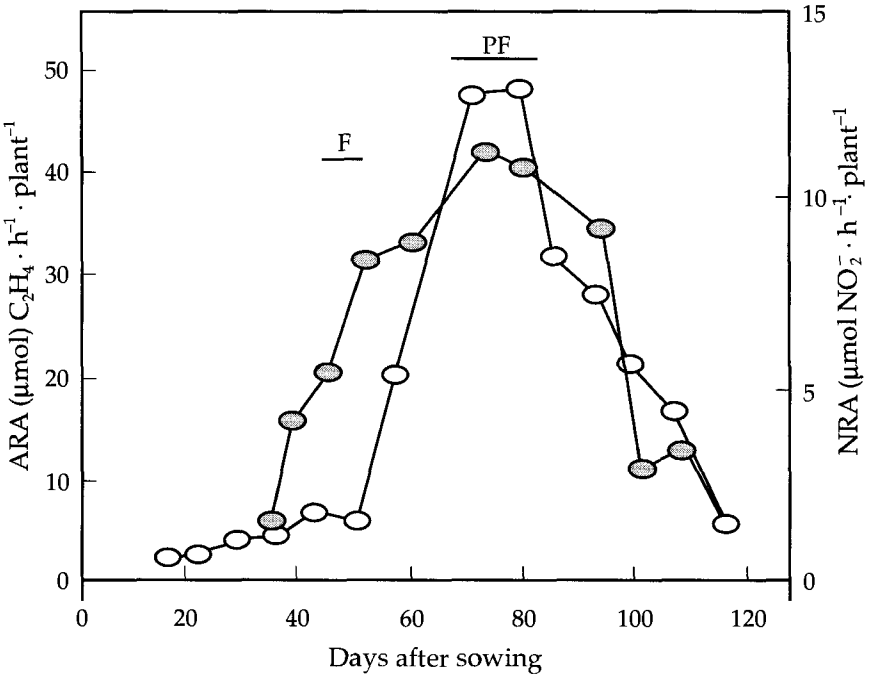
The legume-*Rhizobium* symbiosis imposes additional nutritional requirements apart from the minerals needed for plant growth as a whole. Nutrients that affect SNF include high NO_3^- N, P, B, Zn, S, molybdenum (Mo) and cobalt (Co). Some of these (S, Co, Mo, Zn and Ni) have been extensively addressed elsewhere (Giller 2001). This paper focuses only on nitrate and phosphorus.

Nitrate

Although the problem of high N is usually highlighted as affecting SNF, in the SAT environments where the soils are low in organic matter (less than 1%) and little fertilizer is applied by farmers, high soil NO_3 may not be a critical factor. However, high soil NO_3 could be a limiting factor in the high-input rice-legume-wheat systems. Mineralization of organic matter and nitrification can also result in increased NO_3 concentration in tropical soils. In many semi-arid tropical soils, there is a flush of mineralization of organic matter in the surface soil layers at the start of the rainy season, due to drying and wetting cycles that accelerate mineralization of the labile fraction of soil organic matter, resulting in a flush of mineral N in the top soil layers (Wani et al. 1997). Surveys of farmers' fields in South Asia showed the occurrence of high levels of soil mineral N before sowing of a legume crop (up to 70 ppm in soil surface), which can prevent nodulation and N_2 fixation (Wani et al. 1997). Indeed, high levels of soil mineral N (30ppm) at sowing reduced nodulation of chickpea by at least 14% and the proportion of fixed N by 63%. In the case of pigeonpea, suppression of N_2 fixation was recorded at 43 ppm soil N, and in cowpea at 66 ppm. A direct negative relationship was also observed between soil N levels and nitrogenase activity (Wani et al. 1997).

The inhibition of nodulation and N_2 fixation by combined nitrogen prevents optimal exploitation of both pathways of legume N nutrition (SNF and nitrate assimilation). This inhibition results from complex events occurring at different stages of nodule development (Streeter 1988) and depends upon many factors such as plant genotype, *Rhizobium* strain, and form and concentration of combined nitrogen supply. Inhibition of N_2 fixation by NO_3 is common in all legumes, although it varies between legume species and cultivars (Piha and Munns 1987a, Serraj et al. 1992, Herridge et al. 1994). Most studies to enhance N_2 fixation capacity of legumes in the presence of high levels of nitrate (NO_3- tolerance) have focused on the host plant. This is entirely justified by the results showing limited variation in N_2 fixation in rhizobial strains under high NO_3 conditions (McNeil 1982).

In most grain legumes and cropping situations, SNF alone is not capable of ensuring total N requirement for optimal growth and productivity, which requires a certain level of complementary N delivered through absorption and assimilation of mineral N. In this case, the two principal enzymatic activities responsible for N assimilation, i.e. nitrate reductase activity and nitrogenase activity, could either occur successively or simultaneously during plant development, depending on the level of available soil N. For instance, Serraj et al. (1993) showed that both activities varied in parallel (Fig. 2), which indicated that the two modes of N nutrition could be complementary. The similar patterns of nitrate reductase and nitrogenase activities during the growth cycle of soybean agreed with previous results in lucerne (Wery et al. 1986). However, other reports (Harper 1974, Obaton et al. 1982) showed that both N nutrition pathways were successive during the growth cycle. These contrasting conclusions are likely to be related to the variability of NO_3 level in the medium (Serraj et al. 1993).



Source: From data of Serraj et al. 1993.

Figure 2. Pattern of foliar nitrate reductase activity (open circles) and acetylene reduction activity (filled circles) during the growth cycle of undeterminate soybeans grown in the field. F and PF indicate flowering and pod-filling stages, respectively.

Phosphorus

The N_2-fixing legume plants usually require more P than plants dependent on mineral N fertilizer. Nodule establishment and function are important sinks for P, and nodules usually have the highest P content in the plant (Sinclair and Vadez 2002). Therefore, P deficiency conditions result in reduced SNF potential and P fertilization will usually result in enhanced nodule number and mass, as well as greater N_2 fixation activity per plant.

There are two potential physiological approaches to improving plant growth and yield under low soil P availability (Clarkson 1985), namely (1) efficient uptake of external P, and (2) efficient utilization of internal P. The first approach involves plant-soil interactions such as modification of soil exploration by roots, improved interactions with soil microorganisms such as mycorrhizal fungi, and rhizosphere modification to increase P availability (Ohwaki and Hirata 1992, Hinsinger 1998). The second approach involves efficient partitioning and subsequent utilization of P within the plant, resulting in more biomass produced and more N_2 fixed per unit of P taken up (Föhse et al. 1988).

The P requirements for N_2 fixation have been investigated in various legume crops like cowpea (Cassman et al. 1981), pea (Jakobsen 1985), soybean (Israel and Rufty 1988) and *Acacia mangium* (Ribet and Drevon 1996). These studies show that P requirements are generally higher for N_2 fixation than for shoot growth and mineral N assimilation, since nodules are an additional strong sink for P. Furthermore, P requirement for N_2 fixation has been shown to vary among genotypes in pigeonpea (Adu-Gyamfi et al. 1989) and mungbean (Gunawardena et al. 1992) or Casuarina–Frankia symbioses (Sanginga et al. 1989). Differences in N_2 fixation related to the efficiency of utilization of P were also found among soybean genotypes (Gunawardena et al. 1993) and *Acacia mangium* populations (Vadez et al. 1995). According to Cassman et al. (1981), efficient P utilization in N_2-fixing symbioses may be closely related to an adequate P partitioning between shoot and nodulated root, and between root and nodules.

Long duration legumes having indeterminate growth and low HI have been identified as critical to improving P and N sustainability in smallholder and subsistence agriculture in Africa (Snapp 1998). Intercropping of pigeonpea with cereals is also a proven management scheme for increasing available P. In India, more than 90% of pigeonpea production is in intercropping. And this success is due in part to the exudation of piscidic acid from pigeonpea roots, which enhances the availability of phosphate from iron-phosphate (Ae et al. 1990). Mycorrhizal inoculation of pigeonpea was observed to further improve P uptake in this study. Thus, in addition to providing an immediate source of dietary N, incorporation of pigeonpea residues after seed harvest make P and N more available to a subsequent crop.

Soil Acidity

There are more than 800 million ha of Oxisols and Ultisols in Latin America alone that have a pH less than 5.0. Acid soils pose a major challenge to sustainable agriculture, and particularly to the establishment of N_2-fixing symbioses. SNF can be seriously reduced in such soils, due to the effects of high H^+ concentration, toxic levels of Al and Mn, and induced deficiencies of Ca, P and Mo. Soil acidity limits rhizobial growth and survival in the soils, as well as root nodule development. Growth in acidified culture media has proved useful for selecting strains with an ability to colonize the rhizosphere and nodulate their host plant in acid soils (Cooper 1988). Acidity affects several steps in the development of the symbiosis, including the exchange of molecular signals between the legume and the microsymbiont (Hungria and Vargas 2000). Therefore, nodule formation in many legumes is delayed or inhibited by low pH, lack of calcium, and the presence of dissolved Al. In white clover nodulation is inhibited by Al, even at relatively high pH values (5.5-6.0). Mechanisms governing competition between rhizobial strains for nodule formation under acid conditions are poorly understood and the genetic basis of acid tolerance in rhizobia has yet to be elucidated. Liming is effective in overcoming soil acidity and aluminium toxicity. Selection of rhizobial inoculant strains that are genetically stable under the acid soil conditions is also essential, but this is impaired by a lack of knowledge of tolerance in the microsymbiont.

Large variations in tolerance of acidity factors are found both within and between *Rhizobium* species. Fast-growing rhizobia are generally considered more acid sensitive than *Bradyrhizobium*, but low pH-tolerant strains exist in many species. Variations in acid tolerance within species of root nodule bacteria imply a genetic basis to low pH tolerance and studies of acid-sensitive mutants suggested that a large number of genes and regulatory systems could be involved (Glenn et al. 1998).

In the extensive agriculture of the developing world, it was recommended that the first phase of reclaiming acid soils low in P and N should be the use of a legume cover crop supported by liming and conservative P application (von Uexküll and Mutert 1995). Incorporation of the legume residue will also result in higher soil organic matter content and increased P and N availability (Vance 2001).

Temperature

It has been well documented that both low and high temperature extremes can prevent nodulation, or if nodulation occurs, can inhibit SNF. Rennie and Kemp (1982) studied effects of temperature on nodulation and acetylene reduction in *P. vulgaris*, and showed that both processes occurred at

temperatures as low as 10 °C. At the lower temperatures, the cold adaptability of the plant for early root growth determined its ability to nodulate and fix nitrogen. At higher temperatures, plant growth stage was a determining factor. Inoculation with *Rhizobium phaseoli* at more advanced growth stages decreased the time of nodulation at all temperature treatments, but resulted in higher yield and more N_2 fixation (Rennie and Kemp 1982). Using eleven common bean cultivars representing a wide range of types, and grown in nitrogen-free medium in a controlled environment at two temperature regimes, these authors showed differences between cultivars in their ability to fix N_2 under the two temperature regimes.

Day et al. (1978) reported that in northern Nigeria, rhizobial populations of only 4-40 cells g^{-1} soil were found at the surface (5 cm soil depth), while up to 10^4 cell g^{-1} soils were found at a depth of 20-25 cm below the soil surface, indicating the sensitivity of rhizobia to high temperatures. Tropical legumes have adapted to a wide range of temperatures and there is a wide variability in the ability of different legumes to adapt to different temperatures. In chickpea, N_2 fixation seems to be more sensitive to high temperature stress than seed production and N assimilation (Rawsthorne et al. 1985). Piha and Munns (1987b) have earlier reported the existence of inter- and intra-specific genetic variability in SNF sensitivity to high temperature. Soybean genotypes showed a higher level of genetic variability in their SNF response to high temperature compared to common bean genotypes (Piha and Munns 1987b).

Atmospheric Carbon Dioxide

The increase in atmospheric CO_2 concentration associated with global climate change is now well documented (Allen 1994), and these increases are expected to be even more dramatic in the future (IPCC Third assessment report 2001).

Increased CO_2 concentrations stimulate plant photosynthesis rates, but increases in overall plant growth in the natural environment in response to increased CO_2 are less certain. Plant growth is often limited by factors other than potential photosynthetic rate, of which lack of water and N availability are the two most common (Seligman and Sinclair 1995). It has been hypothesized that legumes might particularly benefit from increased atmospheric CO_2 because their capability of establishing symbioses with N_2-fixing bacteria allows them to minimize natural N limitations to growth (Hebeisen et al. 1997). Legumes have, indeed, been shown to be highly responsive to increased CO_2 under well-watered conditions (Hebeisen et al. 1997, Serraj et al. 1998b). However, the physiological basis of this effect and its relationship with SNF are still unresolved. An early report by Hardy and Havelka (1976) showed that short-term CO_2 enrichment resulted in a significant stimulation of ARA in field-grown soybean. However, the long-

term CO_2 effect promoted nodule growth, but not nodule-specific activity. Similarly, Cabrerizo et al. (2001) recently confirmed that continuous CO_2 enrichment led to increased nodule biomass and carbon availability to nodules but did not enhance specific N_2 fixation in pea.

An important consideration, however, is that N_2 fixation in some legumes is highly sensitive to soil drying (see section on Drought). Because global environment changes associated with increased atmospheric CO_2 are likely to include variable weather conditions, including more frequent and severe episodes of drought, there is the possibility that the importance of the N_2 fixation advantage of legumes in response to CO_2 might be neutralized or completely lost under these circumstances.

Serraj et al. (1998b) showed that exposure of soybean plants to increased CO_2 combined with water deficit treatments resulted in water conservation under both well-watered and drought treatments. It was also discovered that the N_2 fixation activity response to soil drying was greatly altered by increased CO_2. Consistent with earlier observations, N_2 fixation under ambient CO_2 was very sensitive to soil drying and decreased in response to soil drying before the other measured processes (Sinclair et al. 1987, Sinclair and Serraj 1995). In sharp contrast, N_2 fixation became highly tolerant to soil drying under the 700 μmol CO_2 mol^{-1} treatment. Only in the final stage of soil drying when the drought stress was quite severe did N_2 fixation under the 700 μmol CO_2 mol^{-1} finally decrease. These results indicated that the advantage of legumes under global climate change is even greater than anticipated because of the induced increase in N_2 fixation tolerance to drought.

Oxygen Nodule Diffusion and Regulation of SNF

Because N_2 fixation has a high-energy demand, oxygen supply is highly critical in the regulation of nitrogenase activity and N_2 fixation (see review by Minchin 1997). The respiration rates in the nodules must be very high to provide sufficient ATP and reducing capacity. At the same time, O_2 must be maintained at an extremely low concentration in the infected cells to prevent inhibition of nitrogenase. This is made possible by the presence of leghemoglobin and the existence of a variable nodule O_2 permeability (Po) (Minchin 1997). The variable component of nodule Po involves changes in the distribution of air spaces within the nodule internal cortex (Fig. 3), resulting from an occlusion of intercellular space and/or changes in the volume of some of the cells (Walsh et al. 1989).

An osmotic model of regulation of Po in the nodule cortex in response to environmental factors has been proposed by several authors (Witty et al. 1987, Purcell and Sinclair 1994). Both salinity and drought stress, possibly mediated by decreases in phloem flow to the nodules, have been found to affect nodule Po (Serraj et al. 1994, Serraj and Sinclair 1996).

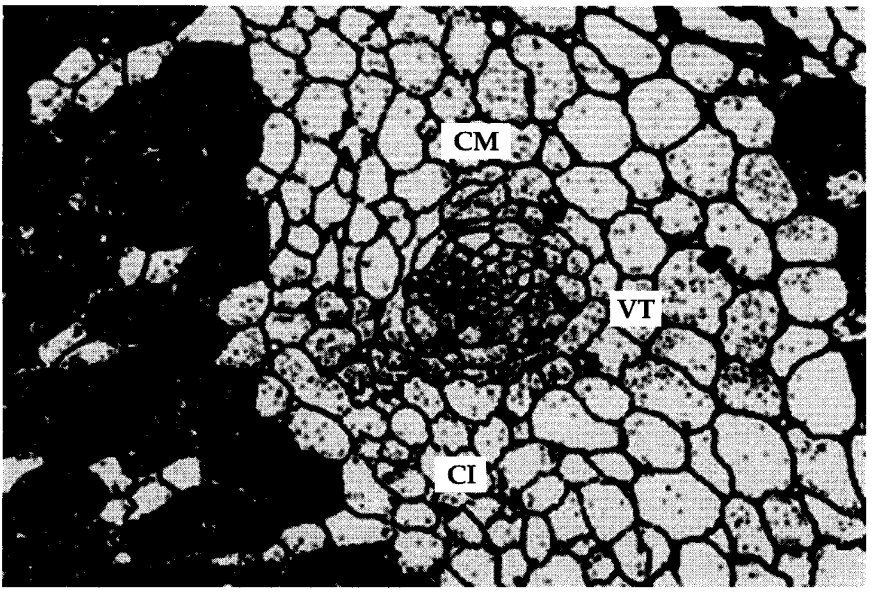
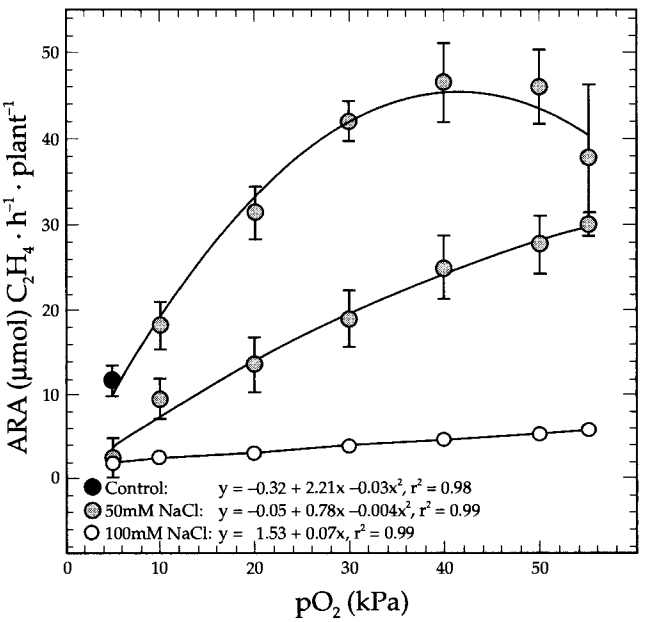


Figure 3. Light micrograph of transverse section of a soybean nodule, (CI, internal cortex; CM, middle cortex; VT, vascular trace).


ARA (µmol) $C_2H_4 \cdot h^{-1} \cdot plant^{-1}$
pO_2 (kPa)
Control: y = –0.32 + 2.21x –0.03x², r² = 0.98
50mM NaCl: y = –0.05 + 0.78x –0.004x², r² = 0.99
100mM NaCl: y = 1.53 + 0.07x, r² = 0.99

Source: Serraj and Drevon 1998.

Figure 4. Effect of NaCl concentration and external oxygen pressure (pO_2) on acetylene reduction activity of alfalfa nodules.

Serraj and Drevon (1998) showed that the responses of alfalfa nodules to rhizosphere external oxygen pressure (pO_2) varied significantly with the NaCl concentrations in the culture medium. Importantly, the inhibition of nodule ARA by 50 mM NaCl was completely reversible by increasing pO_2 around the nodules (Fig. 4), which indicated that an oxygen limitation within the nodules caused by NaCl may have inhibited respiration and nitrogenase activity. When a rapid stress was imposed on soybean plants by adding polyethylene glycol (PEG) in the solution around the roots, it was observed that the decrease in respiration that followed the PEG treatment resulted in a decrease in Po (as calculated from respiration rates) (Serraj and Sinclair 1996). Importantly, the PEG-induced decline in the first hours after treatment was reversible by increasing pO_2 around the nodules, which indicated that an O_2 limitation within the nodules inhibited respiration and nitrogenase activity within the first hours following the PEG treatment. This interpretation agrees with the model of nodule O_2 regulation proposed by Drevon et al. (1995), suggesting that nodule Po is controlled by a mechanism of contraction/expansion of osmocontractile cells in the nodular internal cortex.

In contrast to the ability of increased pO_2 to reverse the decline in N_2 fixation rates in the presence of moderate stresses, nodules exposed to high NaCl concentration did not have ARA stimulated by pO_2 (Fig. 4). These results indicate that nitrogenase activity under this severe stress was constrained by factors other than pO_2. A similar conclusion has been made in the case of drought stress, showing that damage in nodule activity after exposure to severe water deficits was not reversible (Diaz del Castillo et al. 1994, Serraj and Sinclair 1996).

PHYSIOLOGICAL MECHANISMS OF SNF REGULATION UNDER STRESS

Drought

The effect of drought stress on N_2 fixation has usually been perceived as a one-dimensional physiological process acting on nitrogenase activity and involving exclusively one of three hypotheses: Oxygen limitation, feedback regulation by ureides, and carbon shortage. These hypotheses were recently considered together with water transport and nodule structure to be putative mechanisms affecting N_2 fixation in response to drought stress (Serraj et al. 1999b). There is growing evidence for interactions between water and N transport, C metabolism, nodule permeability to oxygen, and nodule growth and function. It was therefore concluded that the various hypotheses proposed so far for effects of drought on N_2 fixation should be integrated into a multi-dimensional model of physiological response to drought.

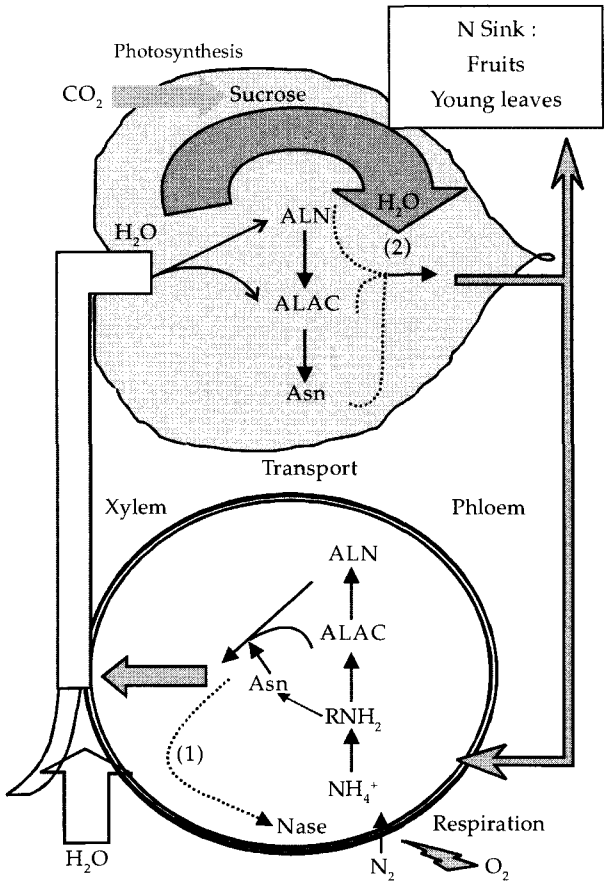
The mechanism by which ureide accumulation may trigger the inhibition of nitrogenase activity is still unclear. The low solubility of ureides (Sprent 1980) may be important in the association of high ureide concentrations with drought sensitivity. The response of N_2 fixation in soybean plants to various nitrogenous compounds further demonstrated the importance of a ureide-related regulation (Serraj et al. 1999a). Plants exposed to 10 mM allantoic acid had decreased ARA and Po within 3 d of exposure, although plants exposed to 10 mM asparagine had a greater decrease in ARA and Po that was initiated after only 2 d of exposure. The severity of the ureide-induced decrease in ARA was dependent on the ureide concentration in the nutrient solution and was partially reversible upon the removal of the ureides from the solution. These results indicated an important role of ureides in influencing nodule activity and Po, but also that asparagine might have a more direct role in this regulation.

Overall, there is now abundant evidence that N feedback on nodule activity is important in the sensitivity of the N_2 fixation response to water deficit conditions (Fig. 5). The feedback in those legumes that export ureides from the nodules seems to be especially aggravated by the accumulation of ureides in the plant. However, the specific feedback signal compound or the mode of action of the feedback is not yet known.

The variation among legume species and cultivars in sensitivity of N_2 fixation to water deficit indicates that the tolerance trait found in some genotypes may be useful in breeding programs for N_2 fixation drought tolerance in legumes. Although the mechanisms of interaction between ureide metabolism and N_2 fixation response to drought are still unknown (Serraj et al. 1999b), measurements of ureide concentration in the petiole and or the xylem sap may be useful in screening large numbers of germplasm of ureide-producing legumes for drought tolerance. Purcell et al. (1998) used petiole ureide concentration measurement to screen a large number of soybean plant introductions, and they found a broad variation in ureide contents. Among the low-ureide producers, they isolated a few soybean lines that showed a substantial level of drought tolerance of N_2 fixation. This observation is important because it indicates that grain legumes can be selected for decreased sensitivity of N_2 fixation to soil drying in regions where drought is a recurring problem.

Salinity

The short-term response of N_2 fixation to salt showed a 2-phase inhibition of nitrogenase activity, i.e. a dramatic decrease in nodule ARA during the first hour of the treatment with NaCl, followed by a slower rate of decrease (Serraj et al. 1994, 1998a). Similarly, Munns (1993) proposed a biphasic model for plant growth response to salinity, the first effect of NaCl being osmotic and



Source: Serraj et al. 2001.

Figure 5. Hypothetical scheme of the relationship between legume nodule and leaf metabolism and possible origins for feedback regulation of nodule activity by N compounds.

the second being toxic ion accumulation (see also Kingsbury and Epstein 1986, Ortiz et al. 1994). Therefore, the initial effect of NaCl on nodule nitrogenase might be caused by a decrease in phloem sap supply to nodules, i.e. a water deficiency, because of an osmotic effect of NaCl at the whole plant level. Fortmeier and Schubert (1995) have suggested a similar mechanism for the inhibition of leaf growth that occurs within minutes after exposure to NaCl (Yeo et al. 1991, Ortiz et al. 1994). The hypothesis of salt inhibition of nodule activity is supported by the similarity of the short-term effects of NaCl and water deficit on nodule activity (Serraj et al. 1994, Serraj and Sinclair 1996).

The existence of inter- and intra-specific variability in the sensitivity of N_2 fixation to salinity has been recently confirmed in legumes (Serraj et al. 1998a). Salt limited nodule growth and nitrogenase activity in soybean, common bean and alfalfa (Serraj et al. 1998a). Exposure to NaCl also resulted in a significant decrease in plant biomass accumulation in common bean and soybean. In contrast, there was no significant effect on biomass accumulation of alfalfa. Although the exposure to salt induced an immediate decrease in nodule N_2 fixation for all symbioses, the rate of inhibition was faster in common bean than in soybean and alfalfa. This level of N_2 fixation sensitivity to salt stress was associated with the level of salt accumulation in the nodules. Exposure to NaCl increased the Na and Cl content of all plant tissues and cultivars, although the content was higher in nodules than in shoot tissues. Common bean nodules accumulated higher NaCl levels than soybean and alfalfa (Serraj et al. 1998a), which confirmed previous evidence showing that salt tolerance in mesophytes was correlated with ion exclusion (Greenway and Munns 1980). Furthermore, legume species and cultivars differ in ion distribution and especially the ratio Na/K within plant organs (Ortiz et al. 1994, Cordovilla et al. 1995). However, little information is available on the effect of salt on ion distribution in legume nodules.

Mineral Nitrogen

The concept of N feedback regulation has been proposed as an alternative general mechanism for the inhibition of N_2 fixation by nitrate and other environmental factors. Silsbury et al. (1986) first suggested a feedback control of nodule activity mediated through the pool of soluble N in the plant. Parsons et al. (1993) developed this concept further by suggesting that nodule formation, nitrogenase activity and nodule permeability to oxygen might be controlled by the concentration of reduced N compounds entering the nodule through the phloem (Fig. 5).

No precise signal molecule for feedback on N_2 fixation under drought conditions has been found so far. However, several reports have shown that free amino acids such as alanine, GABA and proline accumulate markedly in drought-stressed plants and cells (Handa et al. 1983, Rhodes et al. 1986, Raggi 1994). Although the exact physiological significance of such accumulation remains unknown, it has generally been interpreted as an osmotic adjustment mechanism (Handa et al. 1983, Raggi 1994). Bacanamwo and Harper (1997) proposed that the changes in shoot asparagine level and/or products of its metabolism in the nodules might be involved in the feedback control of nodule activity. Baker et al. (1997) suggested that a similar mechanism in *Alnus glutinosa* would involve changes in xylem citrulline. Neo and Layzell (1997) proposed that changes in phloem sap glutamine content may trigger the inhibition of nodule metabolism and nitrogenase

activity. Finally, Vadez et al. (2000) showed that asparagine cannot be the only compound involved in the feedback inhibition of N_2 fixation in soybean, but ureides and asparagine are probably both involved, either directly by accumulation of products that fail to be exported from the nodules, or by feedback from the shoot due to an N-compound supply that exceeds shoot requirements.

P deficiency

Acute deficiency of P can prevent legume nodulation. Work at ICRISAT and elsewhere has shown that legumes like lupin, chickpea and pigeonpea have the ability to extract P from sparingly soluble P sources. Pigeonpea is better able to utilize P bound to the iron fraction of the soil (Fe-P) than chickpea, and soybean, and this explains why the crop responds less to added P than other crops in Alfisols, where Fe-P is high. The carboxylic anions (piscidate, citrate, and malonate) exuded from pigeonpea roots have high P-solubilizing ability. Ishikawa et al. (2002) reported genotypic differences in the P-solubilizing ability of pigeonpea; thus the inclusion of some genotypes of pigeonpea in cropping systems could enhance the available P pool in soils.

Although the understanding at the physiological level has led to the current progress at the molecular level, more work is needed to assess the efficacy of the mechanism of rhizosphere acidification, and carboxylic anion exudation. The genetic manipulation of root exudates is hampered by numerous technical problems in collecting, analyzing and quantifying the exudates. Very little success (if any) has been achieved in measuring root exudates in the soil rhizosphere. In addition, there is lack of a simple, fast and inexpensive technique for the assessment of large numbers of genotypes of a segregation population without using the expensive spectrophotometer. The technique recently reported by Ishikawa et al. (2002) that uses a filter paper qualitative assay method to screen for the P solubilizing ability of pigeonpea will benefit both conventional and genetic engineering approaches to enhancing P use efficiency of legumes. Other aspects of SNF and P acquisition have been extensively discussed recently (Vance 2001, Sinclair and Vadez 2002).

ROLE OF SNF IN TROPICAL CROPPING SYSTEMS

Symbiotic nitrogen fixation accounts for a large proportion of the N currently utilized in agriculture and will be an increasingly important component in future crop productivity especially for sustainable agricultural systems, small-scale operations and marginal land utilization. It is imperative that we not only understand the contribution of this process to various agricultural systems, but we must also appreciate current limitations to SNF under field conditions. Reducing fertilizer use, while maintaining the native soil N

resource and enhancing crop N output is desirable from both environmental and economic perspectives. This may be possible by obtaining more N on the soil through SNF, reducing loss of N and by recycling of N captures in vegetation during the off-season.

Adu-Gyamfi et al. (1997a) have reviewed the dynamics and management of N in sorghum/pigeonpea intercropping systems in the SAT. Their review indicates that the effective management of indigenous soil N and N derived in situ through SNF has the potential to enhance the N nutrition and N use efficiency of crops and the total N output from a sorghum/pigeonpea intercropping. In a four-year study, where the proportion and amount of N derived from air were estimated by both the natural abundance and the relative ureide abundance methods, pigeonpea intercropped with sorghum derived 56-85% N from N_2 fixation, which was more than the %Ndfa by sole crop pigeonpea (32-58%). The amount of N derived from fixation was higher in intercropping than in sole cropping. Data from Adu-Gyamfi et al. (1995) show that the method of fertilization had significant effect on %Ndfa. Highest values were observed for split-banding (87%) compared to broadcasting (67%) in pigeonpea intercrop. In sole crop, the values were 26% for broadcast and 40% for split-band.

From the relative ureide abundance method, the proportion of ureides, amino acids and nitrate (NO_3-N) concentration in xylem sap at the different sampling times were estimated. Nitrate-N accounted for 50-80% of the composition of N solutes in xylem sap exudates in sole crop. The proportion of ureide in xylem sap exudates of sole crop pigeonpea decreased with increased N application. Significantly higher proportion of ureides and amino acids in xylem exudates were recorded for intercropped than for sole crop pigeonpea. Ureide concentration (mM) in the exudates was higher in intercrop pigeonpea than in sole crop; and there was a marked decrease in %Ndfa by sole crop but not in intercropped pigeonpea as fertilizer rates increased.

Intercropped pigeonpea derived about 80% of the N in plant from the air at 65 days after sowing (DAS) compared to 60% for sole crop. The % Ndfa value was higher in intercrop than in sole crop, but significantly lower in delayed than in basal treatments. The % Ndfa significantly increased with DAS.

In a pigeonpea-millet-groundnut intercropping system, higher proportion and amount of N was derived from N_2 fixation compared to the other combinations. These results suggest that more efficient utilization of N can be achieved by appropriate combination of component crops. Intercropped pigeonpea fixed between 80-100 kg ha^{-1}. The intercropped pigeonpea had less opportunity to acquire N from fertilizer and soil compared to sole crop pigeonpea, probably because of the rapid depletion of N by the cereal companion crop, thereby increasing the dependency on SNF (Adu-Gyamfi et al. 1997b, Tobita et al. 1994).

Sanginga (2003) recently reported that promiscuous soybeans were used to develop sustainable cropping systems in the moist savannahs in West Africa, where N has been gradually depleted from soils, causing serious threats to food production. The actual amounts of N_2 fixed by soybeans and their residual N benefits to subsequent cereal crops varied between 38 and 126 kg N ha^{-1}. When only seeds of soybeans were removed from the plots, the net N accrual of soil nitrogen ranged between -8 and +47 kg N ha^{-1} depending on the soybean cultivar (Sanginga 2003). Residual N values of 10-24 kg N ha^{-1} were also obtained in a soybean-maize rotation. These authors also demonstrated that the relative increase in maize N was smaller than the relative increase in dry-matter yield, which indicates that the increased maize yields following soybeans were not entirely due to the carry-over of N from soybean residues, but to other rotational effects as well. It was therefore concluded that the N benefit of grain legumes to non-legumes is generally small compared to the level of N fertilizer use in more intensive cereal production systems but is significant in the context of the low amounts of input in subsistence farming.

CONCLUSIONS: A FRAMEWORK FOR AGRO-PHYSIOLOGICAL CONTRIBUTIONS IN OVERCOMING SNF LIMITATION BY ENVIRONMENTAL CONSTRAINTS

The extreme sensitivity of SNF to environmental and agronomic stress results in a significant decrease of N accumulation in legume crops exposed to these stress factors. Consequently, legume yields are seriously limited under these conditions and most of their potential benefit in the cropping systems is reduced.

It is common knowledge that drought-tolerant varieties in general have low yield potential. Therefore, the degree of drought tolerance incorporated needs to be matched with the magnitude of drought in the target environment. Research should therefore focus on the physiological basis of resilience traits so that systematic efforts might be made to incorporate characters in new varieties. Precise identification of traits is important both in conventional trait-based breeding and in identifying the genetic markers related to the trait.

In conclusion, several interventions should be considered for the agro-physiological optimization of SNF in cropping systems:

- On-farm participatory screening and integration of already identified stress-adapted legume varieties in various crop and crop-livestock systems
- Catalyzing farmer-to-farmer seed diffusion of selected improved legumes tolerant to various environmental stresses, permitting strategic seed reserve development at household level

- Documenting the nodulation status and inventory of current legume varieties tolerant to biotic and abiotic stresses in a target agroecological environment
- Field studies to establish heritabilities of root morphological traits associated with nutrient and water uptake by legumes
- Field studies to evaluate the efficiency of utilization of fixed N by legumes and subsequent crops
- The genetic improvement of legume tolerance to stress, with additional effort for understanding the physiological limitations of SNF at the levels of host plant and rhizobial strains, and their interaction
- Physiological and genetic dissection of traits involved in abiotic stress tolerance among legume species exporting amides or ureides
- Using the information on the genetic variability in P acquisition and nitrate tolerance by legumes to improve P efficiency and develop nitrate-tolerant legume crops
- Identification and evaluation of land management options to increased SNF contribution in cropping systems based on land capability and agro-ecological potential
- On-farm participatory evaluation and adaptation of improved management options for increased SNF contributions in farming systems

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5

Improved Livelihoods from Legumes – A Review of BNF Research at the International Center for Agricultural Research in the Dry Areas

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ABSTRACT

Chickpea, lentil and faba bean (food legumes), and vetch and chicklings (feed legumes) are important cool season crops grown in the WANA region. They are a rich source of protein for human and animal diets, fix atmospheric nitrogen into the soil through their nodules, and play an important role in improving soil, human and animal health. The legumes also play an important role in the mixed farming system where crop-livestock integration is common and households keep livestock to complement crop activities. The work done on different aspects of BNF including the need for inoculation, characterization of *Rhizobium*, *Rhizobium*-cultivar interaction, moisture-BNF relationship, inoculant production, quantification of N_2 fixation and nitrogen balance, rotation of different food and forage legumes with cereals etc., in WANA region has been reviewed in this paper. The studies on rotation of cereals with legumes, use of efficient *Rhizobium* strains, and improving N_2 fixation through appropriate growing season and increased water use efficiency revealed that these legumes can make a major contribution to the 'health' of an agro-ecosystem by improving soil fertility through BNF and sustainability through improved soil structure. The improved methodologies for N_2 fixation and production of these crops will increase availability of these legumes and will thus help in improving the livelihood of the poor in the developing world.

INTRODUCTION

Malnutrition is multifactorial in origin and its basic determinant is poverty (UNICEF 1990, Jonnson 1995). Poor socioeconomic status often leads to inadequate diets in terms of both quality and quantity. According to the

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4th Nutrition Report (ACC/SCN-IFPRI 2000), globally 182 million preschool children are classified as undersized. Another report indicates that about 535 million women are anemic (ACC/SCN 1997). The main cause of malnutrition is lack of food and/or improper dietary diversity (UNICEF 1990, Jonnson 1995). The diets heavily based on cereals and tubers in many developing countries often show nutritional limitations in food energy, including that of protein (Young and Pellett 1994), and a number of micronutrients like iron, zinc and vitamin A (ACC/SCN 1997, Allen 1994).

It is known that the foods that add diversity to the diet and provide most of the essential macro- and micronutrients are animal products (meat, milk and fish). These foods especially serve as sources of high quality protein and essential amino acids such as lysine, which is one of 20 essential amino acids and has been identified to be the key limiting amino acid in the human diet (Pellett and Young 1988, Young et al. 1989, FAO/WHO 1991, Pellett 1996). Animal foods on average contain 85 mg lysine g^{-1} protein, cereals contain only 30 mg g^{-1} protein, and legumes contain 65 mg g^{-1} protein and amino acids.

Legumes are an inexpensive alternative to animal feeds when combined with cereals and/or tubers. Additionally, legumes are dense in micronutrients with iron (50 ppm), zinc (33 ppm) and B-carotene (20 ppm), which improve dietary quality.

In developing countries (Africa, Latin America and Asia) legumes are widely consumed but due to a variety of reasons the availability of what has traditionally been considered "poor man's meat" is low. In a society where access and availability of animal foods in the diet is dependent on socioeconomic status, the poor have limited access to animal products.

Furthermore, the increased population pressure and stagnation of area of the pulse crops has reduced the per capita availability of pulse protein globally from 5 g/capita/day to 4 g/capita/day over the last 25 years. A largely vegetarian country, India had 10 g/capita/day pulse protein availability in 1970, but this declined to 8 g/capita/day by 1995 (Kelley et al. 2000). Thus, improving the availability of legumes as a complementary protein source is of major importance.

Across the developing world, different pulses are consumed in Africa, Asia and Latin America, but in inadequate quantities. Furthermore, each pulse has its individual key limiting biotic and abiotic factor, which keeps production costs high and availability low.

Increased adoption and utilization of legumes can be expected when farmers see clear benefits to their farming systems. In this article we have focused on the input of N into the system. Emphasis also needs to be placed on finding multiple or alternative use of legumes. These could include suppression of weeds, control of pest and diseases by bringing the legumes

as 'break' crops, and substitution of animal protein in human diets particularly for children and women.

In addition to their beneficial effects on human health, legumes make a major contribution to the 'health' of an agro-ecosystem by improving soil fertility through atmospheric nitrogen fixation (N_2 fixation) in association with *Rhizobium* and through improved soil structure.

The postharvest processing of food legumes at the community level and their marketing offers the opportunity to generate cash income. Alternative industrial uses of food legumes will provide avenues of increased income to legume-growing farmers.

The main objective of the CG Centers is alleviation of poverty in the developing world, which needs special consideration when we deal with the legume crops, known as the crops of the poor. The present concept of global challenge programs for agricultural development should address the following points:

- Issues of global importance which may have a regional/ecoregional focus
- Harnessing system-wide energy and encouraging a broader range of external partnerships
- Focusing on and incorporating on-the-ground resources and concepts
- By definition using networking as a tool
- "Open book" approach to diversify the CGIAR research agenda
- Inclusion of broader range of partners for conduct & delivery of research
- Facilitating improved interaction with the private sector on a win-win basis
- Attracting new funding to the system by extending the relevance of CG research to communities beyond the current donor community
- Pragmatic approach that will resolve difficulties experienced by components of CGIAR in functioning as a system

Thus, the alleviation of poverty needs to be addressed as a systemwide approach. It is known that mixed farming systems involving varying degrees of crop-livestock integration are common in developing countries, where households keep livestock to complement crop activities. The International Center for Agricultural Research in the Dry Areas (ICARDA) is mandated to undertake research to improve the productivity of the rainfed farming systems in the dry areas of Central and West Asia, and North Africa (CWANA) in a sustainable manner. Although cereals dominate the cropping system in this region, several food and feed legume crops are also important. These include lentil, chickpea, faba bean, peas, vetches (*Vicia* spp.) and chicklings (*Lathyrus* spp.). In CWANA, for example, legume straw and other by-products of

processing of legumes are often a major source of good quality animal feed. The diversification of farming through the integration of livestock is crucial to the sustainability of rural agriculture, as revenue from the sale of live animals and products would help increase the income of farming households.

One of the major roles that the annual forage and food legumes play in the dry rainfed farming systems (which are invariably subsistent in nature and have little monetary inputs) is the influx of combined nitrogen in the system through BNF. In the Mediterranean region, where most of the cool season annual food and forage legumes have evolved, there has been co-evolution of the micro-symbiont *Rhizobium* along with the macro-symbiont, the host legume plant. However, the association is not always optimal in stressful environments where the micro-symbiont might have evolved more for survival rather than for efficient symbiotic fixation.

The seeds of legumes are a rich source of good quality vegetable protein for human and animal consumption, and the straw serves as nutritious fodder for livestock. In addition, N_2 fixation plays an important role in sustaining the productivity of low-input, cereal-dominated cropping systems in the dry areas of CWANA. The Germplasm Enhancement Program at ICARDA aims at enhancing this role of cool season food and feed legumes through the development and dissemination of improved biological nitrogen fixation (BNF) technology. This report summarizes some of the work done at ICARDA in this area.

COLLECTION AND CHARACTERIZATION OF *RHIZOBIUM*

ICARDA collected the *Rhizobium* spp. from nodulating lentil, chickpea, faba bean, vetches and chicklings from major production areas of WANA, and 255 collections for lentil, 120 for chickpea, 139 for faba bean, and a smaller number for forage legumes were made. Moawad and Beck (1991) studied 229 *Rhizobium leguminosarum* isolates from the lentil-growing areas of WANA region, and evaluated them for their symbiotic effectiveness, and for tolerance to salt and heat. These included 65 isolates from the ICARDA culture collection, strains from international collections, and fresh isolates from soils and nodules of lentil (*Lens culinaris* L.) growing in Turkey, Syria, Jordan and Egypt. *Rhizobium* populations collected from different fields varied from 31 to 690,000 rhizobia g^{-1} soil. The fields from Jordan contained higher average population densities than those from other areas evaluated. The symbiotic effectiveness of all collected isolates was evaluated on lentil cultivar-ILL 16 in an aseptic N-free hydroponics gravel culture system. A greater proportion (up to 50%) of highly effective isolates was found in samples from eastern Turkey and Jordan; samples from Syria and Egypt mainly contained rhizobia with moderate to poor N_2-fixing capacity. Of the isolates collected from all locations, 44% were of low effectiveness: only 21% were classified as superior

N_2 fixers. The *Rhizobium* isolates from Eastern Turkey and Jordan exhibited greater symbiotic efficiency as compared to other populations. Distinct variations in salt and heat tolerance were observed for isolates from different regions. A greater proportion of Egyptian isolates grew at 35 °C compared with international standard strains. Only 9 of 229 isolates grew at 40 °C; 8 of these came from the Southern Nile valley in Egypt. Isolates from Jordan and Turkey were more sensitive to 0.5% NaCl in the growth medium than the others, of which 30-50% were tolerant. Few isolates grew with 1.0% NaCl; most of these came from Syria. None of the heat- or salt-tolerant isolates was among the most effective N_2 fixers.

Materon et al. (1995) surveyed the native rhizobia capable of symbiosis with potential pasture legume crops in the West Asian highlands and estimated the numbers and N_2-fixing efficiency of isolates of *Rhizobium meliloti* with a range of annual Medicago species. Soils were collected from 105 sites at elevations between 500 and 2200 m. They observed that the numbers of bacteria were generally adequate to permit efficient nodulation but the N_2-fixing efficiency of three of the four host species with the indigenous rhizobia was often low. In contrast, the efficiency of N_2 fixation in *Medicago aculeate* was generally higher. No overall geographic pattern in either numbers or efficiency of N_2 fixation was evident. They suggested that substantial research is required before annual Medicago crops can be successfully introduced into highland crop/livestock systems in Turkey and elsewhere in the West Asian highlands.

SCREENING TECHNIQUES FOR EVALUATION OF *RHIZOBIUM* STRAINS

An aseptic hydroponics gravel-culture system has been developed for evaluation of the symbiotic efficiency of the strains. The efficiency is determined on the basis of total plant nitrogen accumulation in inoculated plants grown on nitrogen-free medium as compared to accumulation of nitrogen by plants grown on adequate supply of combined nitrogen in the hydroponics system. The efficient strains identified are then evaluated for their efficacy in intact soil cores, where the selected *Rhizobium* strain is introduced in the presence of native *Rhizobium* population and the nitrogen yield of inoculated plants is compared with those of un-inoculated plants adequately supplied with fertilizer nitrogen.

Finally, the promising strains identified from the intact soil-core system are evaluated under field conditions, generally in combination with a range of promising cultivars, to identify optimum host-genotypes and *Rhizobium*-strain combinations. These procedures have also been extended to NARS for use (ICARDA 1992).

NEED FOR INOCULATION STUDIES

Studies on inoculation with superior strains of *Rhizobium* were conducted in intact soil core plastic house experiments using treatments with and without N, to compare plants reliant solely on N_2 fixed through symbiosis with native rhizobia with plants supplied with ample nitrogen from the soil. During 1986/87, 15 sites in representative chickpea-growing areas in Syria were surveyed. Of these, 7 responded significantly to N and 8 did not. Those responding to N application generally contained less than 500 chickpea rhizobia per gram of soil, and showed a positive response to inoculation with effective competitive strains. Those not responding to N application represented soils containing high populations of effective rhizobia.

Field trials designed to determine the need for inoculation were developed and distributed by ICARDA to cooperators in different countries in WANA as International Fertility cum *Rhizobium* Trials for chickpea, lentil and faba bean. These trials utilized essentially the same methodology as soil core experiments, where nitrogen fertilized plants are compared to symbiotic plants, at two levels of P/K fertility (native and enhanced by fertilizer application). The results communicated by the cooperators indicated a potential response to inoculation in many areas. Where high populations of native rhizobia were present, as in Jinderis and Tel Hadya in Syria, an yield depressing effect or no response was obtained with addition of 100 kg N ha^{-1}. Where local rhizobia existed in lower numbers, such as in Breda in Syria, a significant response to N fertilization was obtained indicating deficiency in the symbiotic system.

RHIZOBIUM STRAIN-HOST CULTIVAR STUDIES

Rhizobium strain-host cultivar studies were conducted on three food legumes (chickpea, lentil and faba bean) and results revealed the significance of interactions depending on the locations (ICARDA 1991). The methodology for such trials included comparison of crop performance using varying treatments including inoculums with a series of effective strains; uninoculated, and uninoculated with 100 kg N ha^{-1} in split applications. In addition, the trials were conducted over four seasons (1987/88 to 1990/91) in northern Syria and N_2 fixation and yield were evaluated with a range of chickpea cultivars inoculated with selected superior *Rhizobium* strains in order to establish baseline values in recommended cultivars. In the non-inoculated treatment, P_{fix} (proportion of crop N derived from N_2 fixation) remained at about 60% when the dry matter yield ranged from 2 to 7 t ha^{-1}. The effect of this constant proportion of crop N derived from fixation means that with increasing dry matter (and N) production, the quantities of soil N taken up by the crop increase. In contrast, with *Rhizobium* inoculation, the efficiency

of N_2 fixation increased at higher yield levels, reaching a maximum of 80%. Increased fixation efficiency with increased yield resulted in an increasing proportion of fixation-derived N in the plant and a lower, relatively constant amount of soil-derived N.

The inoculation studies with different strains of *Rhizobium* using a range of chickpea cultivars showed a positive interaction of inoculation with seed and nitrogen yield responses as well as for the fraction of total nitrogen derived from fixation. This suggested that through appropriate genotype and *Rhizobium* strain combinations BNF could be considerably increased. Thus, improvements through rhizobial strain selection and legume breeding can be quantified (Beck 1992).

NITROGEN RESPONSE AND BALANCE USING DIFFERENT LEGUMES

Nitrogen balances of four legume crops for two cropping seasons were studied at Tel Hadya, Syria and for one season at Montpellier, France during 1986/87 and 1987/88 (Table 1). Orobanche, a parasitic weed, severely affected lentil, faba bean and peas in the 1986/87 season at Tel Hadya, whereas a cold winter in Montpellier reduced the differences in response between winter and spring planting in chickpea.

In another collaborative study with ENSA-INRA, Montpellier, France, the proportions of plant N derived from N_2 fixation in four grain legume crops (faba bean, chickpea, lentil and pea) were estimated in 3 field experiments conducted over two seasons in Syria, and one in France. Since cultural practices and cultivars affect grain yield, the impacts on N_2 fixation of sowing date in chickpea, and Sitona control in lentil, and of cultivar selection in pea and faba bean were evaluated (Beck et al. 1991). By calculating the proportion of total plant N derived from fixation (% Ndf) using ^{15}N isotope dilution with barley and non-nodulating chickpea as reference crops, the effects of removal of N in grain and straw, relative to N_2 fixed and plant uptake of soil mineral N were estimated. Pea and lentil had similar % Ndf values across locations, seasons, and cultural practices, with an average 70% Ndf. In chickpea, winter sowing increased % Ndf to 72% (from 26% in spring-sown chickpea). BNF in spring-sown chickpea was higher in France (44% Ndf), while fixation in winter chickpea was higher in Syria (80%). Faba bean obtained 90% Ndf in France but only 69% in Syria. The calculated N balance where only grain was removed ranged from 44 kg N ha^{-1} net gain in large seeded faba bean to 44 kg N ha^{-1} net loss in spring sown chickpea in France. Where both seed and straw were removed, nearly all calculations were negative, with losses of up to 70 kg N ha^{-1} from soil (Beck et al. 1991). These results indicated that the N balance using different legume crops was influenced greatly by the crop and the end use product. Grain production

Table 1. The biological yield (BYLD), grain yield (GYLD), total crop nitrogen, % nitrogen from fixation, kg N ha^{-1} from fixation, and kg N ha^{-1} from soil of four legume crops for two cropping seasons at Tel Hadya, Syria, and one season at Montpellier, France.

	Location	Season	BYLD (kg ha^{-1})	GYLD (kg ha^{-1})	Total crop N kg ha^{-1}	% N from fixation	kg N ha^{-1} from fixation	kg N ha^{-1} from soil
Winter chickpea	Tel Hadya	1986/87	5988	2407	181	70	127	54
	Tel Hadya	1987/88	6079	2441	112		78*	33*
	Montpellier	1986/87	4974	3327	130	60	78	52
Spring chickpea	Tel Hadya	1986/87	1226	545	21	42	9	12
	Tel Hadya	1987/88	2251	960	36		18*	18*
	Montpellier	1986/87	4526	3102	120	47	57	52
Lentil	Tel Hadya	1986/87	6020	1126	130	68	88	42
	Tel Hadya	1987/88	7009	2354	132		90*	42*
	Montpellier	1986/87	7926	3515	215	79	169	46
Faba bean	Tel Hadya	1986/87	4562	1742	118	76	89	29
	Tel Hadya	1987/88	7518	3289	17		135*	42*
	Montpellier	1986/87	5873	3614	195	94	183	12
Pea	Tel Hadya	1986/87	1458	751	44	72	32	12
	Tel Hadya	1987/88	4018	1814	86		62*	24*
	Montpellier	1986/87	7289	3427	171	82	141	30

*Estimated using the 1986/87 values for %N from fixation

may increase or deplete the soil-N pool, depending on the ratio of N_2 fixed to seed N. In the WANA region, the legumes are often harvested by hand, and the straw is utilized as animal feed or for fuel as in the case of lentil. Removal of all above ground plant material in case of lentil will deplete soil N and may have negative effect on the N nutrition of a subsequent crop, but some cultural practices like winter sowing of chickpea can increase grain yield and N balance.

BIOLOGICAL NITROGEN FIXATION BY COOL SEASON LEGUMES AND ITS IMPACT ON WHEAT PRODUCTIVITY IN SYRIA AND LEBANON

Trials to investigate the role of both food and forage legumes in rotation with wheat through contribution of BNF were initiated during the 1990-91 season at two locations varying in rainfall (Tel Hadya in Syria and Terbol in Lebanon) using a 2-course rotation system (ICARDA 1993). To differentiate between the applied nitrogen and fixed nitrogen, varying treatments were used including ^{15}N application, and use of non-nodulating chickpea and barley as reference crops. Continuous wheat and fallow-wheat over a longer period allow an evaluation of the contribution of legumes to N inputs in a farming systems approach. Both phases of the rotation were grown each year. Phase I included legume treatments, where the quantities of N fixed were measured using ^{15}N methodology. Phase II was planted with wheat, in which varying levels of N fertilization allowed calibration of N contribution from Phase I treatments against yield and N-uptake from added N fertilizer. From Phase I treatments, N contributions from soil and fixation in the legume crop were obtained, from which the potential N contribution to soil was calculated. With Phase II treatments, fertilizer use efficiency (FUE) and the amount of fixed N from the previous crop treatment (Soil A value) were measured using ^{15}N enrichment data.

The results for the period 1991-1994 from Tel Hadya and Terbol (Table 2) indicated that forage legumes were in general more efficient than grain legumes in their BNF, although the actual values are greatly affected by environmental conditions (Table 3). Highest yields of fixed nitrogen were around 100 kg N ha^{-1} in Terbol and nearly 60 kg N ha^{-1} in Tel Hadya, obtained with *Vicia villosa* ssp. *dasycarpa*. Wheat yields following the legumes were at par with yield of wheat following fallow and significantly higher than the yield of wheat following wheat.

EFFECT OF MOISTURE SUPPLY ON N_2 FIXATION

Since the rainfall in the WANA region is highly variable and available moisture is one of the limiting factors for productivity of the crops, studies

Table 2. Percentage of plant nitrogen derived from fixation (%Ndf Fix) in different legumes at Terbol (TR) and Tel Hadya (TH) stations.

Treatment	%Ndf Fix							
	1990/91		1991/92		1992/93		1993/94	
	TR	TH	TR	TH	TR	TH	TR	TH
1. Lentil	72.5	72.5	81.2	72.4	82.4	62.7	78.2	81.0
2. Lentil(+Promet)	78.1	78.2	85.7	72.3	85.3	69.9	71.8	87.9
3. Winter chickpea (-)	57.8	53.2	61.9	67.4	-	52.5	68.0	75.3
4. Winter chickpea (+Strain CP-39)	56.5	71.8	68.2	67.4	-	43.6	64.3	74.0
5. Spring chickpea (-)	55.6	64.2	32.1	63.7	-	50.2	-	66.1
6. Spring chickpea (+CP-39)	51.1	59.8	35.3	70.8	-	40.8	-	61.3
7. Faba bean	75.0	79.0	-	80.0	-	82.4	81.1	87.6
8. Dry peas	66.5	75.3	73.0	71.3	84.4	59.4	65.0	81.6
9. Dry peas(+Promet)	68.2	79.0	73.9	73.5	85.2	82.2	73.4	81.9
10. *Vicia dasycarpa*	71.2	81.5	77.4	72.9	88.0	72.4	79.4	88.8
11. *Vicia narbonensis*	71.2	74.5	82.2	72.8	87.7	77.4	71.2	83.5
12. *Vicia amphicarpa*	-	-	-	-	-	-	-	47.1
13. *Lathyrus sativus*	78.9	74.4	83.7	73.0	87.9	67.9	75.3	82.6
SE(d.)	6.4	7.8	6.9	9.2	5.1	10.5	5.9	6.8
LSD(5%)	13.2	16.2	14.3	19.1	11.0	21.8	12.4	14.1
CV(%)	12	13	12	16	7	20	10	11

Table 3. Weather conditions at Tel Hadya (Syria) and Terbol (Lebanon) during four seasons, 1990-1994.

	1990/91	1991/92	1992/93	1993/94	Long-term averages
A. Tel Hadya:					
Seasonal rainfall (mm)	290	353	290	373	328
Number of frosty days	35	57	48	19	–
B. Terbol:					
Seasonal rainfall (mm)	531	860	664	475	550
Number of frosty days	53	72	91	66	–

have been undertaken to investigate the effect of variable moisture supply on the productivity and BNF in lentil and chickpea.

The effect of rainfall and production methods on crop productivity and BNF was studied in 1981/82 at 8 different locations in northern Syria chosen to span the large isohyetal gradient (200-600 mm seasonal precipitation) experienced in this region (Keatinge et al. 1985, 1988). At each site, three crops (lentil, chickpea, faba bean) and different treatments were selected in accordance to local practice. ^{15}N technique was used to determine BNF using barley as the non-fixing reference crop. Varying treatments were used to differentiate between the applied nitrogen and fixed nitrogen, including ^{15}N application, non-nodulating chickpea, and barley as a reference crop. Results revealed that reduced seasonal rainfall (from over 300 mm to close to 200 mm) resulted in low BNF (falling from values around 50-100 kg ha^{-1} to below 20 kg ha^{-1}). However, the improved agronomy (P application, use of efficient strain of *Rhizobium*, optimum date of planting, weed control, etc.) significantly improved the dry-matter production and BNF in most of the site treatment combinations.

Studies were conducted during 1988/89, utilizing the line-source sprinkler and ^{15}N-enriched micro plots at the Breda site of ICARDA with 6 lentil lines (ICARDA 1991), and these revealed large effects of moisture supply on % Ndf – it doubled from an average 36% at lowest moisture supply (180 mm) to 72% at moderate moisture supply (330 mm), and showed a marginal increase of 5% (from 72% to 77%) when moisture supply was further increased (to 376 mm). This implied that the symbiotic system in lentil lines used in this study was near maximum efficiency and there may be little chance to further improve %Ndf with increased moisture level.

Similar studies using six cultivars of chickpea during 1988/89 sown in spring at Tel Hadya under three moisture levels revealed that the average %Ndf (based using six cultivars) increased with increasing moisture supply, 19% at 290 mm moisture, 42% at 407 mm, and 64.5% at 449 mm (ICARDA 1991). These studies also indicated that further increase in moisture supply might increase % Ndf.

A comparison of N_2 fixation values for lentil and chickpea with regard to increasing yield associated with increased moisture supply showed that the nitrogen economy of the system was better at higher moisture supply with chickpea than lentil, because chickpea was able to have an increasing proportion of its total nitrogen coming from fixation while lentil showed no such increase (ICARDA 1994).

PRODUCTION OF INOCULANT

In view of the fact that inoculation with efficient strains of *Rhizobium* has been found to increase the yield of food and forage legumes in several areas of the ICARDA region, there is a need to encourage the production of high quality inoculums. Its unavailability or high cost hinders the use of peat as a carrier for *Rhizobium* inoculants in many countries. The capacity of soil to support the survival of rhizobia suggests that mineral soils, particularly if amended with organic carbon, could substitute for peat. Beck (1991) studied the ability of two soils, with or without amendments of wood charcoal, to support prolonged rhizobial survival as compared to high quality Australian peat. In a series of three experiments, soil amended with charcoal proved equally effective at maintaining high ($>10^9$ g^{-1}) populations of rhizobia nodulating chickpea (*Cicer arietinum* L.) for periods of 105-126 days. After storage for 280 days, two *Rhizobium* strains differing in growth rate maintained viable numbers in the soil-charcoal mixture above l08 g^{-1} indicating the suitability of this material as an inoculant carrier. The results suggest that high-quality *Rhizobium* inoculants may be produced with some mineral soils and locally obtained materials where peat is not available.

NITROGEN FIXATION AND N-BALANCE

An understanding of the magnitude of N_2 fixation and its contribution to plant N, particularly in harvested grain and straw, is necessary to assess the potential of grain legumes to contribute to long-term agricultural production stability.

Biological nitrogen fixation was estimated for different food legumes under rainfed conditions of Tel Hadya in 1980/81 and 1981/82 seasons using ^{15}N methodology (Saxena 1988). BNF yield ranged from 75 kg ha^{-1} in winter chickpea to 107 kg ha^{-1} in lentil treated with Carbofuran to protect nodules from damage by the larvae of Sitona weevil in 1980/81 (total seasonal rainfall was 350 mm). In 1981/82 when the total seasonal precipitation was somewhat lower (338 mm) and cold was more severe, the BNF yields were lower (ranging from 27.9 kg ha^{-1} in the winter chickpea to 80.7 kg ha^{-1} in pea).

The residual effect of the treatments during 1980/81 season on the grain yield of rainfed wheat was studied in 1981/82, when wheat was fertilized

with 3 levels of nitrogen (0, 30 and 60 kg N ha^{-1}) to permit assessment of the 'nitrogen' and/or other effects of the previous season's legumes. The yield of wheat following lentil, faba bean, and dry peas was significantly higher than that following wheat at all levels of fertilizer N, although the differences were higher when no nitrogen was applied. Assessment of the soil 'A' value nitrogen in 1981/82 wheat crop revealed that there was decided "enrichment" of nitrogen status of the soil following the legumes in comparison to those following wheat (Pala et al. 1994). The nitrogen status of this soil was extremely low and the wheat crop following wheat showed severe nitrogen deficiency at 0 and 30 kg N ha^{-1}.

Thus, the studies conducted in West Asia and North Africa revealed that BNF by different legume crops varied widely at different sites. *Rhizobium* surveys conducted in different areas further exhibited the presence of wide genetic variability among different *Rhizobium* strains present in each crop. The incorporation of legumes in rotation with cereals resulted in improvement of soil health and productivity of the farming system. The BNF efficiency of a crop could be increased by improving the water use efficiency, and by use of appropriate efficient *Rhizobium* strain. In conclusion, the legumes can play a significant role in improving the sustainability of the cereal based farming system by breaking the monotony of cereal cultivation, and improving the soil and human health, and ultimately, the livelihood of the poor.

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6

Biological Nitrogen Fixation: A Key Input for Integrated Soil Fertility Management in the Tropics

CIAT-TSBFI Working Group on BNF[1]

ABSTRACT

This paper describes the importance of biological nitrogen fixation (BNF) by legume-*Rhizobium* symbiosis to tropical agriculture, the evolution of BNF paradigms, creation of strategic alliances to combat soil fertility degradation, and accomplishments of collaborative BNF-related research at CIAT-TSBFI. It suggests that a holistic-multidisciplinary-systems approach is needed to integrate BNF-efficient and stress adapted legumes into smallholder systems. It proposes a number of research and development priorities for achieving improved BNF contributions through integrated soil fertility management, a holistic approach to soil fertility that includes all driving factors and consequences of soil degradation. Although BNF has not proved a solution for strain selection or breeding of host, modest progress has been registered. The technology is economically viable. The environment is at least as limiting on BNF as is the strain and the host. The benefits of BNF are best expressed in the context of an agronomic management system that addresses other components of the crop, especially P supply, drought stress and frequently, starter N. Selection for BNF capacity under physiological stress has revealed genotypes worth exploiting more fully. Research efforts on BNF in tropical forage legumes indicated that the main constraints to their widespread adoption include a lack of legume persistence, presence of anti-quality factors such as tannins, variable *Bradyrhizobium* requirements, and a lack of acceptability by farmers. Farmer-participatory selection of legumes for increased acceptability is needed. Substantial progress was made in creating an organic resource database and using it to construct a decision support system for organic matter management. Analysis of organic resource data indicated a set of critical values of nitrogen, lignin and polyphenol content for predicting the "fertilizer equivalence" of organic inputs. This provides

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farmers with guidelines for appropriate use of organic materials for soil fertility improvement. The paper also suggests key interventions that are needed to achieve greater impact of legume-BNF technologies.

INTRODUCTION

It is widely recognized that biological nitrogen fixation (BNF) by legume-*Rhizobium* symbiosis is an important component of productivity in tropical agriculture, especially in farmland that is marginal either in terms of distance from the markets, or small farm size and the poverty of the farmers (Giller 2001). In such resource-poor, smallholder systems the application of large quantities of inorganic fertilizers such as urea is not economically feasible. The use of management techniques that increase the contribution of N to the system through the legume-*Rhizobium* symbiosis would improve crop-livestock production levels and their stability. A major challenge for BNF research is developing strategies to integrate BNF-efficient and stress-adapted legumes (grain/forage/green manure/cover/fallow) into local cropping systems for the crucial transition of smallholders from subsistence agriculture to mixed-enterprise, market-oriented production systems. It is only through this development that spiraling declines in poverty, food insecurity and land degradation may be addressed.

Although significant advances were made in BNF research during the 20th century, the impact of this research on improving productivity of smallholdings in the tropics through N input has been small, less than 5 kg N ha^{-1} yr^{-1} (Giller 2001). Recently, Giller has put forward the view that the BNF in tropical agriculture could be increased enormously if current understanding was put to more effective use via simple agronomic on-farm practices. Beyond this, the most rapid additional gains are likely to come from adapting legume germplasm to different agroecological niches in cropping systems. Other approaches such as genetic engineering are likely to take much longer to yield benefits.

This position paper describes the evolution of BNF paradigms, importance of legume-BNF to tropical agriculture, progress in creation of strategic alliances to combat soil fertility degradation, and past accomplishments of BNF-related research at CIAT-TSBF. Based on lessons learned, the paper proposes a number of research needs and challenges for achieving improved BNF through integrated soil fertility management (ISFM) in the tropics.

Importance of Legume-BNF to Tropical Agriculture and Soil Fertility

Various BNF technologies addressing the problems of food insecurity, poverty and land degradation can be identified with various potentials for BNF (Table 1). Legume-*Rhizobium* symbiosis can sustain tropical agriculture at

Table 1. BNF interventions for income generation and food security, their social benefits, target systems and potential impact.

BNF Interventions	Social benefits (0, 1 to 5)					Land use system	Geo. range	Pot. BNF (kg ha^{-1})	Current BNF (kg ha^{-1})	Pot. impact	Specifics
	Income generation	Food security	Land restoration	Carbon offsets	Biodiversity						
Crop-related											
Soybean rotation (Parasitic weed supp.)	5	2	3	1	1	S to L	SA to H	150	< 50	High	Germplasm imp. Agronomic practices
Cowpea rotation/int	3	4	3	1	3	S to L	SA to SH	70-80	<40	High	P inputs Screening germplasm Marketing Postharvest
Groundnut rot/int	3	3	2	0	1	S to L	SA to H	80	~ 60	High	
Pigeonpea int.	2	3	4	2	1	S	SA to SH	150	<50	Medium-High	
Phaseolus beans int.	3	4	0	0	2	S	SA to SH (MA/HA)	70	<10	Medium	
Livestock-related											
Woody fodder banks	4	2	4	3	2	S	MA to HA	300	30-50	High	*Calliandra* etc.
Herbaceous fodder banks	3	2	3	2	2	S to L	SA to SH	150	50	High	*Stylosanthes,*
Dune stabilization	0	0	5	5	3	Waste	A	120	60	High	*Casuarina*
Woodlots	3	0	5	4	2	Degraded	SA to SH	150-300	50	High	*Acacia* spp
Afforestation	0	0	5	5	4	Low N soils	SA to SH		50		Numerous sp
Woody fallows	1	0	4	3	2	S to L	SH to H		50		P solubilization
Herbaceous fallows	1	0	4	3	2	S to L	SH to H	200	50	Medium	*Mucuna, Pueraria, S. rostrata*
Mixed woody /herbaceous	1	2	4	4	4	S to L	SH to H	300		Medium	Numerous
Woody parkland	1	0	2	2	2	S to M	SA to SH	100	50	Med	[thorny] *Acacia* spp.
Boundary trees	1	0	3	3	2	S to L	SH to H	60	30	High	Numerous

(Adapted from AABNF 2001); 0 = no benefits; 5 = very high benefits; Land use systems: S=small land holdings; L=large holdings; W=wasteland; Geographical range: SA=semi-arid; SH=subhumid; H=humid; A=arid; MA=mid-altitude; HA=highland; BNF reported in kg ha^{-1}/crop for annuals and kg ha^{-1} yr^{-1} for perennials.

moderate levels of output, provided all environmental constraints to the proper functioning of the symbiosis have been alleviated (see later). Legumes can accumulate up to 300 kg N ha^{-1} in 100-150 days in the tropics (Table 1).

Legume-cereal intercrops or rotations are widely practiced in the tropics to minimize the risk of crop failure and to provide households with improved diets. Traditionally, the main contribution of BNF in these systems is to improve household food security and human nutrition rather than improved soil fertility. Table 1, however, indicates various other niches for legumes in cropping systems, each with their own specific contributions to improvement of food security, or land restoration.

Evolution of BNF Paradigms

The African Association of Biological Nitrogen Fixation (AABNF 2001) summarized the first paradigm for BNF research of the 20th century as *"the upper limits of BNF may be steadily increased by the collection and evaluation of ever-more effective N_2-fixing microorganisms and their hosts because the distribution of this elite germplasm will necessarily accrue benefits following their introduction to production systems."* However, greater knowledge over time was not accompanied by improved BNF in the field. The widening gap between scientific advances in BNF and opportunities realized from their application has led to the evolution of a new paradigm for BNF research in the 21st Century: *"research in biological nitrogen fixation must be nested into larger understandings of system nitrogen dynamics and land management goals before the comparative benefits of N_2 fixation may be realistically appraised and understood by society as a whole."* It is critical to note that this does not reduce the importance of N_2-fixing organisms and their products, but rather repositions them from a central autoecological focus into a more integrated component of a larger, more complex task. The rationale behind this new paradigm is that it is not biologically-fixed nitrogen alone which sets the standard for successful contribution to social needs, but rather the products realized from more resilient and productive ecosystems that are strengthened through BNF.

TSBFI-CIAT

The former Tropical Soil Biology and Fertility Programme (TSBF), an international institution devoted to ISFM research, has joined with the International Center for Tropical Agriculture (CIAT) to form the TSBF Institute (TSBFI) of CIAT. This brings together TSBF's expertise in ISFM with that of CIAT in soils and land management as well as the complementary areas of germplasm improvement, pest management, GIS and participatory research. This merger builds on the strong collaboration between CIAT and TSBF in

soil fertility research in East Africa that has developed within the CGIAR Systemwide Program on Soil Water and Nutrient Management (SWNM).

ISFM is the adoption of a holistic approach to soil fertility that embraces the full range of driving factors and consequences – biological, physical, chemical, social, economic and political – of soil degradation. This approach is very closely related to the wider concepts of Integrated Natural Resource Management (INRM) and represents a very significant step beyond the earlier, narrower, nutrient replenishment approach to soil fertility enhancement.

Strategic Alliance to Combat Soil Fertility Degradation through a Holistic Approach

Soil fertility degradation is one of the major constraints to food security in developing countries, particularly in Africa. Despite proposals for a diversity of solutions and the investment of time and resources by a wide range of institutions, it continues to prove a substantially intransigent problem. The rural poor are often trapped in a vicious poverty cycle between land degradation, lack of relevant knowledge or appropriate technologies to generate adequate income, and opportunities to overcome land degradation. CIAT, TSBF and the World Agroforestry Centre (ICRAF) have formed a strategic alliance, the goal of which is 'to improve rural livelihoods in Africa through sustainable integrated management of soil fertility' (Fig. 1). The three partners have made significant contributions to combating soil fertility degradation over the past decade. The alliance will go further by building on existing networks and partnerships to implement a fully integrated program of research and development activities. This alliance is regarded as the first step in a wider partnership consistent with the process of integration of international and national agricultural research activities.

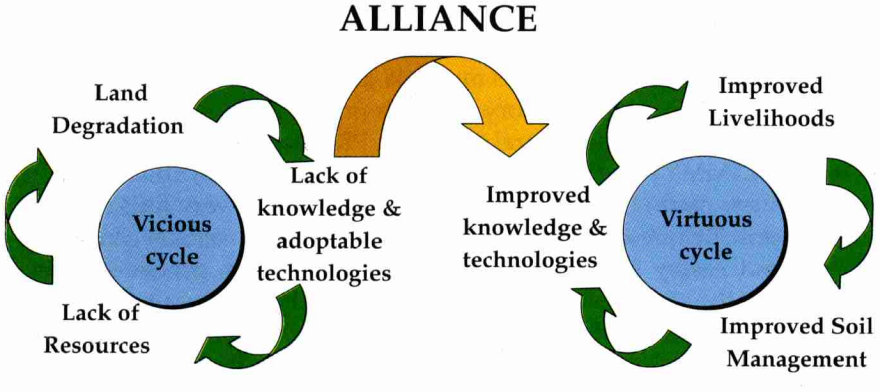

Figure 1. Combating soil fertility degradation: generating ISFM knowledge to improve rural livelihoods.

Ecoregional Alliance on Legumes

CIAT, the International Center for Agriculture in the Dry Areas (ICARDA), ICRISAT and the International Institute of Tropical Agriculture (IITA) formed an ecoregional alliance in 2000, bringing together over 65 qualified scientists working on various aspects of legume production and utilization (genetic resources and breeding, agronomy and microbiology, soil and water management, plant protection, quality and postharvest processing, and socioeconomics). This alliance sees achieving synergy in legume research as a key opportunity to make progress in improving food security, combating environmental degradation and alleviating poverty in developing countries.

Systemwide Program on Soil, Water and Nutrient Management

SWNM is a systemwide global program of CGIAR created in 1996 to help multiple stakeholders rise to the challenge to reverse degradation of soils through the development of sustainable practices for managing soil, water, and nutrients. The SWNM program operates through four complementary research consortia (combating nutrient depletion, optimizing soil water use, managing sloping lands for erosion control, and integrated soil management), and has developed a series of decision support tools and methodologies that are being tested across the different regions in Africa, Asia and Latin America covered by the program. This program could serve as an important vehicle to test, promote and deliver BNF-efficient legume technologies to farmers in the tropics.

Systemwide Program on Participatory Research and Gender Analysis (PRGA)

PRGA is a CGIAR systemwide program on participatory research and gender analysis for technology development and institutional innovation. The program develops and promotes methods and organizational approaches for gender-sensitive participatory research on plant breeding and on the management of crops and natural resources. PRGA is cosponsored by CIAT, ICARDA, CIMMYT and the International Rice Research Institute (IRRI). A recent review carried out by the PRGA program found very little relevant experience in ISFM research with regard to gender-related needs or constraints (Kaaria and Ashby 2001). This lack of a client-oriented, gender sensitive approach to the basic design of ISFM technologies has contributed not only to poor adoption but also to inequity. Therefore, the PRGA is currently supporting research to test novel approaches to pre-adaptive research for ISFM, incorporating client-oriented participatory research methods, such as gender and stakeholder analysis, into very early stages of technology design.

PRGA currently supports research on gender-differentiated approaches to developing technology for integrated nutrient management being conducted by CIAT's participatory research team. PRGA and ICRISAT conducted a study on impact of participatory methods in the development and dissemination of legume soil fertility technologies and identified lessons that will be useful in BNF work (Snapp 1998, 1999a, b, Snapp et al. 2002, Johnson et al. 2001). TSBFI is a partner in implementation of a subsequent project on the use of participatory approaches in research on natural resource management to improve rural livelihoods for women farmers in risky environments.

BNF-RELATED RESEARCH ACCOMPLISHMENTS OF CIAT-TSBFI ON GRAIN LEGUMES AND MULTIPURPOSE LEGUMES

BNF research at CIAT started in the 1970s and the Center maintains a collection of 5,628 *Rhizobium* strains. Several scientists have developed practical ways to enhance BNF in legumes.

Grain Legumes

Genetic Improvement of BNF Efficiency in Grain Legumes: Common Bean as a Case Study

BNF research in common bean (*Phaseolus vulgaris* L.) has spanned the range of strain selection, host improvement, agronomic management, and the recently initiated QTL (quantitative trait loci) studies (Graham 1981, Graham and Temple 1984, Kipe-Nolt and Giller 1993, Kipe-Nolt et al. 1993). The studies on the bean thus illustrate both some of the successes and failures of BNF research. An important attribute of common bean, justifying its inclusion in low-input systems, is the ability to fix atmospheric N and thereby reduce the depletion of soil resources. Beans in tropical environments are capable of fixing from 50 (CIAT 1987) to 80 kg N ha^{-1} (Castellanos et al. 1996). Yet, actual N_2 fixation in bean cultivars is generally low when compared with many other grain legumes. Early research in the late 1970s indicated that this poor BNF is not due to an intrinsic inability of beans to nodulate, as profuse nodulation can occur in controlled conditions in the greenhouse and in some soils. Although poor nodulation is frequently observed, soils in most bean-growing areas contain large numbers of compatible and effective rhizobia. Selection of adapted *Rhizobium* strains for beans sown directly in pots of soils containing large populations of indigenous, compatible rhizobia has resulted in yield increases when these strains were subsequently tested in the field.

Graham and coworkers field tested more than 600 cultivars of common bean under short-day subtropical conditions and found greatest N_2 fixation

in the indeterminate, climbing beans (Graham and Rosas 1977, Graham 1981, Graham and Temple 1984). A very active program of breeding for improving BNF in beans (crossing and recurrent selection) in the early 1980s in small-seeded bush beans generated a number of advanced lines (designated as RIZ lines). Field evaluation of these RIZ lines in the late 1980s in Colombia indicated that they generally nodulated better and fixed more N_2 than their parents (Kipe-Nolt and Giller 1993). However, when compared with other CIAT bred lines, RIZ lines were no better in N_2 fixation than some other lines that were not specifically bred for BNF potential, in particular BAT 477 (see below). A major lesson learned from this breeding effort was that the field sites used for breeding – for better BNF – in Colombia are rich in N; thus the selection pressure was not adequate. These results are in contrast to field evaluation efforts for bean germplasm on infertile soils in Africa, which were remarkably successful in identifying several genotypes with superior adaptation to low N supply (Wortmann et al. 1995). These genotypes improved grain yield on farmers' fields, at least in part due to superior BNF.

Research during the late 1970s and most of the 1980s indicated that environmental constraint(s) limit N_2 fixation in the field. Phosphorus deficiency – which affects 60% of bean growing area – was considered the main factor limiting N_2 fixation in the field. In the early 1990s, specific research into P x BNF interactions in beans was conducted in close collaboration with Institut National de Recherche Agronomique (INRA), France. Extensive effort has been dedicated to seeking sources of bean germplasm tolerant to low P with regard to BNF and to identifying the respective genes. The selection parameter used in breeding for greater BNF was total N accumulation. This work resulted in identification of cultivars and strains that fix N more efficiently in low P soils. Among them, BAT 477 is an unusual bred line in several respects. It is one of the most widely adapted drought-tolerant lines found to date. It has demonstrated unusually high general combining ability among lines within the race Mesoamerica. With regard to BNF potential, it is one of the best N_2-fixing genotypes under unstressed conditions in different soil types as well as stressed conditions of both drought and low P. This suggests that the BNF genes of BAT 477 are especially stable, and are therefore of particular interest for intensive study, and for deployment in bean cultivars.

In the late 1990s, recombinant inbred lines (RILs) of BAT 477 x DOR 364 were used to identify QTLs for BNF under low P stress conditions in collaboration with INRA (Ribet et al. 1997, Valdez et al. 1999). Results obtained indicated that most QTL contributing to greater total N and/or dry weight (DW) proceeded from BAT 477 in the F5 generation, although one QTL that contributed to total N proceeded from DOR 364. It is no surprise that, for a trait as ubiquitous in *Phaseolus vulgaris* as is N_2 fixation, some positive QTL

are found where not expected. Yet, in its development, BAT 477 was never consciously selected for N_2 fixation.

In the late 1980s to early 1990s, a collaborative program between CIAT and NARS to select bean rhizobial strains adapted to specific areas and cultivars was successful in Cuba and Cajamarca, Peru. In Cuba it has been possible to reduce N applications on bean by 80% through inoculation, and a BNF 'package' of strain, genotype and low levels of P inputs gave yields equal to the standard variety with high inputs. The most productive strains are now produced commercially and used by farmers in these two countries (Cuba and Peru). In the majority of cases, however, successful inoculation response trials in Latin America and Africa have been sporadic at best. But in Central America a regional collaborative project tested the benefits of inoculation with selected strains and found an average of 14% yield increase over 39 trials. CIAT maintains a rhizobial strain collection and database. In the early 1990s, research on *Rhizobium* focused on two activities: (1) evaluation of strain N_2 fixation effectiveness and strain x cultivar interactions; and (2) evaluation of factors affecting rhizobial competitiveness. The latter was approached through development of strains genetically transformed to express glucuronidase in nodules, enabling easy wide-scale analysis of inoculation events. This work aimed to identify strains capable of high levels of N_2 fixation across a broad range of cultivars and a high degree of competitiveness under prevalent environmental constraints. CIAT has developed a group of 20 strains transformed with the *gus* gene while maintaining the symbiotic and competitive characteristics of the wild type. These genetically modified strains could serve as valuable tools to evaluate competition × environment interactions.

Another valuable tool that was developed in the 1990s was a series of non-nodulating lines. Mutagenesis was employed to create a mutant with a total lack of nodules. The non-nodulating gene in turn was backcrossed into a series of elite lines, to have at hand a ready tool for estimating the amount of N_2 fixation in any given situation, by comparing non-nodulating and wild type paired lines.

Lessons Learned

In summary, BNF has not been a remedy, either on the side of strain selection or breeding of the host, but modest progress has been registered. On the one hand, even if response to inoculation is not dramatic, the technology is so inexpensive that responses at all levels could be economically viable. On the other hand, the environment is at least as limiting on BNF as is the strain and the host. Therefore the benefits of BNF are best expressed in the context of an agronomic management system that addresses other components of the crop, especially phosphorus, drought and not infrequently, starter N. Selection for

BNF capacity under physiological stress has revealed genotypes (and possibly genetic systems) that are worth exploiting more fully and that could hold keys to broader progress.

Tropical Forage Legumes

Selection of Rhizobial Strains and Development of BNF Technologies for Forage Legumes

BNF research on tropical forage legumes began in the late 1970s and continued throughout the 1980s and 1990s (Date and Halliday 1979; Sylvester-Bradley et al. 1983, 1988, 1991; Sylvester-Bradley 1984; Thomas 1993, 1995; Thomas et al. 1997). Taking into account the wide range of forage legume genera being evaluated, about which very little information concerning BNF was available, the main priority was initially to determine the need to inoculate. After improving the methodology for evaluation of need to inoculate, specifically by ensuring that the presence of mineral N was not interfering with the evaluations, by using different methods to immobilize mineral N, it was found that a surprisingly large proportion of the legumes responded to added N. This indicated that the naturally occurring rhizobial populations were inadequate, either numerically or in N_2-fixing capacity, under the given soil conditions. A program was developed whereby *Rhizobium* strains which (1) were able to compete with the native rhizobial population and (2) would be effective on as wide a range of legume species as possible, were selected. A new method for strain selection, i.e. the screening of large numbers of strains in undisturbed soil cores, was developed, and proved highly successful. Many statistically significant responses to rhizobial inoculation in the field were obtained.

With funding from the United Nations Development Programme (UNDP), a network of scientists was established in the mid-1980s to evaluate legume-*Rhizobium* symbioses in 14 countries of Latin America. Its findings were brought together at a workshop held at CIAT in 1987 where appropriate strain recommendations were made, and continue to be revised as a result of field evaluation by network members. The marked responses to rhizobial inoculation observed in these trials led to the realization that a new way of inoculating the seeds of legumes was needed, so that the technology would be more available to farmers. The UNDP funded project demonstrated that freeze-dried inocula could survive for several years in vacuum-sealed vials and that they could be suspended in water and applied to the seeds with high survival rates. This technology could well be a realistic alternative for supplying forage legume seeds and rhizobial inocula to farmers.

A Swiss Development Corporation funded project demonstrated the need to maintain adequate levels of both P and K for legume-based pastures that

rely on biologically fixed N_2 to supply the N requirement of the pasture (Cadisch et al. 1989, 1993).

CIAT researchers demonstrated fungal/bacterial inhibitory role of *Bradyrhizobium* strains isolated from tropical forage legumes and cell-free culture filtrates of three strains of *Bradyrhizobium* (Kelemu et al. 1995). In vitro screening of 15 strains of *Bradyrhizobium* from the CIAT collection showed that *Bradyrhizobium* could inhibit mycelial growth, reduce or prevent sclerotial formation, and inhibit sclerotial germination in *Rhizoctonia solani*. The antifungal/antibacterial property may increase the competitiveness of *Bradyrhizobium* strains and enhance the chance of nodule occupancy and other beneficial responses with compatible forage legumes.

Role of Legume BNF in Crop-Livestock Systems (Latin America)

As the objective of selection for improved N_2 fixation was mostly achieved, research in 1990s broadened from N_2 fixation per se to the role of the legume and N in productive and sustainable pasture and crop-pasture systems (Thomas 1992, 1995). This work showed that tropical forage legumes have the capacity to meet the requirements to balance the N cycle of grazed pastures. It also showed that the actual amounts required depended on the rate of pasture utilization and the efficiency of recycling via litter, excreta and internal remobilization. The efficiency of N_2 fixation (% of legume N derived from fixation) was found to be usually high in tropical pastures (>80%) and is unlikely to be affected by inorganic soil N in the absence of N fertilizer application. This work resulted in a recommendation that an estimate of the amounts of N_2 fixed by tropical forage legumes could be obtained from simple estimates of legume biomass provided tissue levels of P and K are adequate for plant growth.

The long-term crop-pasture rotations experiment in tropical savannas of Colombia indicated that N recovery by crops from residues was low (7-14%) while recovery from fertilizer was far greater (26-50% in biomass) (Friesen et al. 1998). Sequential measurements of soil profile mineral-N concentrations indicated a large accumulation of nitrate (NO_3)content to 1-m depth through the dry season and substantial NO_3 movement through the soil profile during the wet season under both rotations and monocultures. Thus in the high leaching environments of the humid tropics, poor N supply-demand synchrony can result in substantial leaching of NO_3 below the crop rooting zone and eventual contamination of the groundwater. Use of deep-rooted crop, forage and fallow components could minimize N losses from legume-based systems in the tropics.

Lessons Learned

It was realized that the main constraints to the widespread adoption of forage legumes include a lack of legume persistence, the presence of anti-

quality factors such as tannins, variable *Bradyrhizobium* requirements, and lack of acceptability by farmers. But 'lack of legume persistence' is not really a limitation if the seed is cheap enough. The legume seed can be broadcast into an already established pasture.

Organic Resource Database and Organic Matter Management

In areas where farmers cannot afford adequate quantities of mineral fertilizers, organic sources of nutrients of animal and plant origin, such as legumes, will continue to be a critical source of nutrients (Palm et al. 1997). Organic materials influence nutrient availability (1) by nutrients added, (2) through mineralization-immobilization patterns, (3) as an energy source for microbial activities, (4) as precursors to soil organic matter, and (5) by reducing the P sorption of the soil. The TSBF-SWNM (CNDC) organic resource database (ORD) has been used to construct a decision support system (DSS) for organic matter management based on nitrogen, polyphenol and lignin contents. Most studies indicated a linear response between N content and fertilizer equivalency values (FEQ) of the material with an increase of 8% FEQ for every increase of 0.1% N. In a recent study evaluating FEQ of *Tithonia diversifolia*, *Tephrosia*, *Sesbania* and pigeonpea, yield increases up to 48% were recorded. This decision tree provides farmers with guidelines for appropriate use of organic materials for soil fertility improvement. Ongoing TSBF network experiments are now addressing the organic/inorganic nutrient interactions to allow the refinement of the recommendations to farmers. A systematic framework for investigating the combined use of organic and inorganic nutrient sources includes farm surveys, characterization of quality of organic materials, assessment of the FEQ value based on the quality of organics, and experimental designs for determining optimal combinations of nutrient sources. The desired outcome is tools that can be used by researchers, extensionists and farmers for assessing options of using scarce resource for maintaining soil fertility and improving crop yields (Palm et al. 1997). With the recent success of CIAT scientists with their partners in linking of the DSSAT crop models with the CENTURY soil organic matter (SOM) model (Gijsman et al. 2002), the nutritive value of organic substrates for crop production can be analyzed under a range of climatic and soil conditions and for many different crops. The combined DSSAT-CENTURY also proved to be an excellent tool for evaluating the SOM pattern under low-input systems.

A combination of resource flow mapping, ORD, and FEQ has helped farmers to identify options for enhancing farm productivity and sustainability. Analysis of organic resource data indicated a hierarchical set of critical values of nitrogen, lignin and polyphenol content for predicting the 'fertilizer equivalence' of organic inputs. TSBF and CIAT with a wide range of partners

are also developing methods for disseminating ISFM options through processes of interactive learning and evaluation among farmers, extensionists and researchers.

Legumes in Smallholder Systems in Africa: Lessons Learnt From Experiences of Other Institutes and Initiatives

The potential for legumes is increasing for many smallholder farming systems in Africa as soil fertility declines and livestock management is intensified. Wortmann and Kirungu (2000) summarized lessons from several cases where legumes have been promoted for soil improvement or forage. The cases included *Mucuna* in Benin, *Sesbania* and *Tephrosia* in Zambia, *Calliandra* in Kenya, improved fallows and green manures in Rwanda, *Stylosanthes* in West Africa, *Tephrosia* in eastern Uganda, best-bet niche options in central and eastern Uganda, and *Lablab* in western Kenya. These cases included those where the practice was well adopted by farmers, as well as cases of unconfirmed promise, and adoption failure.

Over 15 years of work in West Africa with leguminous trees in alley cropping systems and *Mucuna* cover crops has led to a series of conclusions. Such systems *are* technically sound and *do* maintain crop yields at substantially higher levels than traditional cropping systems. However, their adoption by farmers is relatively low or absent because (1) the appropriate niches for such systems were not properly identified (e.g., alley cropping must be targeted to high population density areas where firewood is needed and fertilizer is not easily available) and (2) resource-poor farmers require immediate benefits besides improved soil fertility.

As a result of these developments and maybe due to the existence of crop improvement and resource management programs in the same Institute, dual purpose grain and fodder legumes have been developed at IITA that improve the soil fertility status besides providing grains and fodder. Such legumes usually have a large proportion of N derived from the atmosphere, and a low N harvest index, and produce a substantial amount of above ground biomass. Residual effects on a cereal crop are often dramatic and fertilizer use to a subsequent cereal can be cut by 50% while still producing similar maize yields as a fully fertilized maize crop. Furthermore it was found that, e.g., soybean and cowpea could be false hosts for *Striga hermonthica*. One dual-purpose soybean variety, TGX-1448-2E, was specifically appreciated by farmers in Northern Nigeria, who commented that this variety yields more and produces more biomass than their own varieties. In addition, their succeeding maize/sorghum crops gave good yields with less N fertilizer than they would normally apply. The highest net benefits for the two seasons (US$ 1450) were obtained with the rotation of TGX 1448-2E followed by the local variety Samsoy 2 (US$ 1000). The

lowest net benefits (US$ 600) were obtained with *Lablab* (Sanginga et al. 2001).

NEED FOR A MULTIDISCIPLINARY SYSTEMS APPROACH TO IMPLEMENT AN ISFM AGENDA IN THE TROPICS

BNF can contribute directly to the needs of a growing crop or to soil fertility. For sustainable agriculture in the tropics, there are two options: inorganic N fertilizers, or BNF technologies that are less dependent on external purchased inputs. Approaches relying purely on external inputs are not often feasible, particularly for resource-poor farmers of the smallholder systems. In Africa, where the price of inorganic fertilizers is several times higher than world price, alternatives to inorganic fertilizers are especially important. A consensus has emerged that systems of ISFM are the only way forward, and it is in this context that the inputs from BNF must be considered (Fig. 2).

The decision by farmers to adopt ISFM is influenced by (and influences) a range of factors that can be grouped in four main dimensions: biophysical, economical, social and policy (Kaaria and Ashby 2001). The biophysical dimension influence on farmers includes the basic characteristics of the BNF technologies as well as the overall quality of the resource base. The main economic factor that influences whether farmers practice ISFM is whether the economic benefits outweigh the costs, especially in the short term. ISFM/BNF technologies are often labor intensive and if labor costs are too high – or the technologies come at the wrong time of the year when farmers are busy with other activities – then farmers cannot profitably adopt them. Often labor-intensive practices like ISFM are only profitable when used with high value commercial crops. The social dimension also influences adoption and impact of ISFM. Where crop production responsibilities (and rights) are gender-specific, ISFM technologies need to be consistent with these; e.g., work schedules appropriate for women. Legumes can have important human health benefits, although care must be taken to ensure that foods are properly prepared and culturally appropriate (if people won't eat them then maybe they can be used as animal feed). Finally, a supportive policy environment is key to achieving widespread adoption. Fertilizer prices should be rational (not subsidized or taxed) and reflect real costs. This is the best way to ensure that farmers use the right combinations of organic and inorganic soil fertility management practices in their technologies. In addition, property tenure security is important to realize benefits of long-term investments, land ownership, or long-term rental/use arrangements. Infrastructure investments such as roads and communications that open up marketing opportunities can help make adoption of ISFM profitable.

Legume BNF can be a key input to ISFM. When legume BNF technologies are appropriately designed taking into consideration the incentives provided

by each of these four dimensions, they could have positive impacts in each dimension as well. Legume-BNF technologies can improve the sustainability of crop-livestock systems (biophysical), improve profitability, and contribute to improved nutrition and gender equity (social). At the macro level, increased use of legume-BNF technologies could reduce use of costly imported inorganic fertilizers (policy).

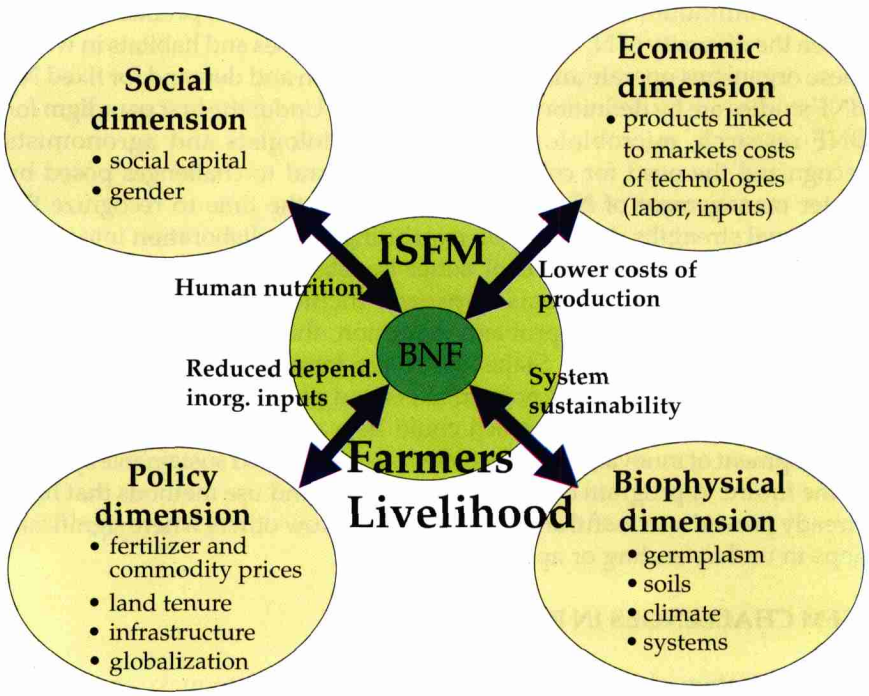

Figure 2. The key role of legume-BNF in the overall integrated soil fertility management (ISFM) strategy.

Most tropical soils have low inherent fertility and exhibit a variety of edaphic and climatic constraints including water stress, nutrient deficiency, low organic matter, and high erodibility. Inadequate soil and crop management has exacerbated these problems to an alarming extent. Because of insufficient levels of nutrient replacement for that taken in harvest and other losses, high negative nutrient balances are commonly reported, particularly in sub-Saharan Africa.

Intensification of agricultural production on smallholdings is required to meet the food and income needs of the poor, and this cannot occur without investment in soil fertility management. This is necessary to help households

mitigate many of the characteristics of poverty, for example by improving the quantity and quality of food, income and resilience of soil productive capacity. The effects of soil fertility degradation are not confined to the impact on agricultural production. The living system of the soil also provides a range of ecosystem services that are essential to the well-being of farmers and society as a whole.

BNF-related research should proceed along the process-component-systems continuum and lead to demand-driven, on-farm problem solving. Given the diversity of N_2-fixing organisms, symbioses and habitats in which these organisms operate and the wide application and demand for fixed N_2, BNF studies are by definition multidisciplinary. Under the first paradigm for BNF research, microbiologists, plant physiologists and agronomists recognized the need for collaboration to respond to challenges posed by better management of N_2 fixation, and now is the time to recognize the additional strengths derived from expanding this collaboration into wider interdisciplinarity as a means of better translating research findings into social benefits. The systems approach includes the involvement of stakeholders to fine-tune problem definition, the research itself, and the implementation of results. Stakeholders are farmers and citizens on farm and community levels, and policymakers and planners at higher levels. A comprehensive systems approach could be a necessary condition for the development of innovative, BNF-efficient, legume-based sustainable systems of the future. A program of work must build on and use methods that have already proved successful and develop and borrow others where significant gaps in understanding or application occur.

ISFM CHALLENGES IN RELATION TO BNF

Implementation of ISFM strategies on farms is likely to make the biggest contribution to agricultural sustainability in the tropics during the coming decade. When combined with robust, highly productive crop varieties, it is not uncommon for such systems to double yields in farmers' fields. The use of improved varieties is an integral part of the ISFM approach; ISFM is a specific strategy under the overall INRM research framework that aims at lifting the barriers between crop improvement and natural resource management. A vital aspect of these strategies is the incorporation of farmers' indigenous knowledge at an early stage of systems development to enhance the adoption of ensuing technology.

Considerable evidence exists that farmers have accumulated knowledge relevant to agronomic management (Carter and Murwira 1995, Murage et al. 2000). Encouraging as this is, increasing land degradation, often including substantial soil fertility decline, suggests that locally devised methods alone are no longer effective enough to cope with rapidly changing pressures on

farmers (Johannes and Lewis 1993, Pinstrup-Andersen and Pandya-Lorch 1994, Murdoch and Clark 1994).

Farmers generally possess a vast body of knowledge about environmental resources in their farms but this is largely based on observable features (Talawar and Rhoades 1998) rather than generalized knowledge. There is a general lack of process-based knowledge about agroecosystem function which is needed to cope with change, especially since much of it is unprecedented (e.g. climate change). This is in particular true for colonist farmers (Muchagata and Brown 2000). In essence, lack of knowledge creates uncertainty that obstructs sound decision-making under conditions of change. This uncertainty about agroecosystem function prevents farmers from taking decisions that are too risky, and may have contributed to their reputation of being averse to risk. However, recent research points out that scientific knowledge can reduce farmers' decision-making uncertainty by enhancing local knowledge (Fujisaka 1996). Some examples already exist that show how this can have positive synergistic effects for agroecosystem management (Steiner 1998, Norton et al. 1998, Robertson et al. 2000).

Research Needs

The holistic systems approach of ISFM is needed to address the smallholder to medium-scale farming sector throughout the diverse agroecological zones of the tropics. This systems approach does not exclude process and molecular studies, but rather suggests that these tools be focused upon recognized constraints within farming systems. Research efforts on legume-BNF related aspects thereby become tools toward a larger purpose, particularly in achieving food security and improving the diets of poor people in the tropics.

Evaluating Genetic Diversity to Overcome Environmental Constraints

Environmental factors affect BNF via growth and development of the host plant, the bacteria and also the process of interaction between the symbionts from the time of infection through the development of the nodules to the production and transport of products. Identification of the processes that are most sensitive to environmental constraints promises the greatest success in breeding programs or in an improvement of agronomic practices (Rao 2001). The major environmental factors affecting BNF in the tropics are drought, soil acidity, soil nutrient deficiency and soil salinity. As substantial genetic variability in tolerance to most environmental constraints exists in both host legumes and rhizobial strains (Hungria and Vargas 2000), there is potential for breeding and selection for improved genetic adaptation. Significant gains in impact can be achieved in the short to medium term by taking advantage of the huge legume and *Rhizobium* genebanks in participatory field evaluation and identification of stress-adapted legumes to specific ecological niches.

Drought

Drought significantly affects BNF in legumes. Decrease in soil moisture causes a rapid decline in the numbers of rhizobia in soil. However, *Bradyrhizobium* strains are more tolerant of desiccation than strains of *Rhizobium* over short periods (Bushby and Marshall 1977). Rates of N_2 fixation by legumes are more sensitive to reduction in soil moisture content than other processes such as photosynthesis, transpiration, leaf growth rates or nitrate assimilation (Serraj et al. 1999). Ureide-exporting legumes with determinate nodules appear to be more sensitive to drought than amide-exporting legumes (Serraj et al. 1999).

Given the increase in occurrence of drought at an alarming rate, especially in sub-Saharan Africa, and the need for incorporation of legumes into systems to improve soil fertility, there is a real need to improve the drought resistance of N_2-fixing legumes. The tools now available in genetic engineering offer a good opportunity to improve drought resistance, making use of the existing genetic diversity. CIAT has been working on development of drought-resistant bean varieties, and identified resistant materials like BAT 477, to be used as genetic sources. A drought protocol was also recently developed for improvement of the genetic adaptation of beans in Africa (Amede et al. 2002). A possible strategy in the short term could be improving water-holding capacity of tropical soils by increasing soil organic matter content and rate of water infiltration while reducing run-off and soil erosion. As most grain legumes in the tropics are grown as intercrops or relay crops, selecting best companion crops and adjusting the planting dates could minimize water stress effects on BNF.

Soil acidity

Soil acidity is increasing in the humid and subhumid tropics, mainly caused by improper land use and high rainfall intensity that encourages leaching of cations. The effects of soil acidity and the associated Al (aluminum) toxicity and P deficiency on BNF could be minimized through increasing the rhizosphere pH. One immediate option is liming but this is beyond the reach of resource-poor farmers, particularly in Africa. There is a consensus that continuous cultivation of legumes over long periods could lead to soil acidification. Therefore, crop rotation or intercropping legumes with cereals (maize-bean or sorghum-cowpea) is one sustainable strategy to improve BNF. Moreover, there are some tropical legumes that produce root exudates (mucilages and organic acids) that could minimize the effects of soil acidification through complexing Al ions. Other potential strategy is to identify legumes less sensitive to Al toxicity. Bean researchers at CIAT are breeding for improved Al resistance. The Eastern and Central Africa Bean Research Network (ECABREN) in Africa has identified bean materials that are less sensitive to Al toxicity when grown in the acidic soils of Democratic

Republic of Congo. CIAT researchers in collaboration with NARS partners have selected a number of tropical forage legumes with very high adaptation to acid soils of the tropics (Rao 2001).

Soil nutrient deficiency

As mentioned earlier, the most limiting nutrient for BNF is known to be P, which becomes limiting in most tropical soils not only for legumes but also for all other crops. The P deficit in soils of the tropics is the result of the combined effect of low inherent P content, very high P fixation, and limited application of soluble P (Rao et al. 1999). Some legumes (e.g. pigeonpea, chickpea) are much more efficient at utilizing P in P-fixing soils, mainly through release of organic acids that increase its availability. Moreover, ECABREN identified bean materials that are performing well under low N, low P and low pH soils of Eastern and Central Africa, indicating genetic difference in nutrient use efficiency. Other institutes are working with P efficient cowpea and soybean (Sanginga et al. 2001).

Soil salinity

Legumes that are grown in the drought-prone environments of sub-Saharan Africa, with saline or sodic soils, are commonly exposed to salt stress. Soil salinity could affect BNF through induction of water stress, pH effect, direct effect of Na ions, or a combined effect. However, the rhizobia were more tolerant than the host plant. Since the initial effect of salt stress is commonly expressed as water stress, improving the soil water availability would improve salt resistance of both grain and multipurpose legumes. Another strategy is integration of well-adapted N_2-fixing perennial legumes to reduce soil pH through acidification.

Breeding/Selection for Improved BNF Efficiency Using Conventional and Molecular Approaches

As mentioned before, one of the bred lines of beans, BAT 477, is not only BNF-efficient but also well adapted to major abiotic stress factors such as water stress and low P availability in soil. What is the probability that independent genes control tolerance of BNF to different stresses; that still other genes control BNF in stress-free environments; and these have come together in one genotype without any conscious selection? This is unlikely. Rather, the same genes probably confer high BNF under all these conditions. In this case, what mechanism could explain the tolerance of these genes to at least two stress factors? The BAT 477 genes may be regulatory genes that are less sensitive to an internal stimulus that results in down-regulation and are thus less active in regulating BNF. Thus, they confer high BNF under a wide range of conditions. It is significant that some QTL, which were tagged in BAT 477 under low P stress, also contributed to better BNF with high P

supply, suggesting that the corresponding alleles in DOR 364 (less adapted to low P supply) may not be expressed fully, even in optimal environments. Could gene regulation therefore limit BNF under optimal conditions? This hypothesis represents a different perspective on what restricts BNF in common bean. There is a need to investigate to what extent the poor BNF of common bean in fact reflects internal limitations of gene regulation.

Identification of Niches Within Cropping Systems

Legumes do occupy space and time in cropping systems and consequently, suitable temporal and spatial niches need to be identified within farming systems for widespread adoption by farmers. Temporal niches are defined by sequential or simultaneous occurrence of legumes while spatial niches are defined by the optimum location to plant legumes, based on farmers' production objectives. The latter often include under-utilized spaces on farms, such as field boundaries, contour strips, or degraded fields. Snapp et al. (1998) identified six temporal niches for legumes. Spatial niches are also related to the existence of within-farm soil fertility gradients, created by inherent soil properties but more often by deliberate land management by the farmer. Such gradients are very often linked to farmers' wealth, and the overall socioeconomic environment (e.g., access to input and output markets, credit schemes for inputs, etc.).

Proper Legume Management

Even nutrient use efficient and promiscuous legume germplasm requires proper crop management for optimal contributions of BNF. To alleviate P constraints to BNF, the simplest option is to apply soluble P fertilizer. In absence of such resources, another possible strategy is through application of rock phosphate. Preliminary evidence shows that certain legumes can immediately access P from nonreactive rock phosphates, where cereals do not have that ability (Vanlauwe et al. 2000a). Proper targeting of P in legume-cereal rotations has also been shown to significantly enhance the growth of maize after application of rock phosphate to herbaceous legumes (Vanlauwe et al. 2000b). A last alternative to alleviate P stress would be through application of farmyard manure, which often contains considerable amounts of available P.

Even where N is concerned, except for the most efficient N_2-fixing legumes, there is often a need to supply starter N especially for those legumes growing in low fertility soils. In the multiple cropping systems of the tropics, it is possibly only the homestead, the most fertile corner of the farm, that may not require external P inputs and/or starter N because of continual application of farmyard manure and household residues.

Appropriate Integrated Nutrient Management (INM) Strategies

The efficient use of fixed N incorporated in the legume biomass is the net result of the dynamics of N in the system and is affected both by intrinsic characteristics of N sources (legume residues, N fertilizers) and N sinks (crop uptake, soil N pools), and by environmental factors (temperature, soil moisture, rainfall intensity and distribution, etc.) that govern process rates. The decomposition and N release rates of crop residues and green manures depend on their composition (ratio of C:N and content of lignin and polyphenols), as well as soil temperature and moisture and the interaction of residues with soil (affected by management) (Palm et al. 2001). Nitrogen derived from organic sources that is not taken up by the crops or incorporated in the soil organic matter pool may be lost from the system through volatilization, denitrification, and leaching. Improving synchrony of crop demand with the rate of legume residue decomposition is therefore of fundamental importance for the efficient use of N from leguminous green manures, covers and residues.

Within the INM framework, it is now recognized that both organic and mineral inputs are necessary to enhance crop yields without causing the soil resource base to deteriorate. This recognition has a practical dimension because one or the other of these inputs is usually unavailable in sufficient quantities to the small-scale farmer. At the same time, it has an important resource management dimension as there is potential for added benefits created by positive interactions between both inputs when applied together. Such interactions can lead to improved use efficiency of the nutrients applied in organic or mineral form or both (Vanlauwe et al. 2001). Two sets of hypotheses can be formulated, based on whether interactions between fertilizer and OM are direct or indirect. For N fertilizer, the *Direct Hypothesis* may be formulated as: *Temporary immobilization of applied fertilizer N may improve the synchrony between the supply of and demand for N and reduce losses to the environment.* Obviously, residue quality aspects will strongly determine the validity of this hypothesis. The *Indirect Hypothesis* may be formulated for a certain plant nutrient X supplied as fertilizer, as: *Any organic matter-related improvement in soil conditions affecting plant growth (except the nutrient X) may lead to better plant growth and consequently enhanced efficiency of the applied nutrient X.*

Due to the complexity involved, the efficient use of participatory approaches in the early pre-adaptive stages of BNF research will ensure that BNF technologies are client-oriented and respond to the needs of farmers and other end-users. Farmer-participatory research (FPR) is increasingly receiving considerable recognition in both international and national agricultural research and development organizations as an important strategic research issue, vital to achieving impacts that benefit poor people

in marginal, diverse and complex environments. There is now a large body of literature that demonstrates considerable advantages and potentials of involving farmers in the research process. FPR can significantly improve the functional efficiency of formal research (better technologies, more widely adopted, more quickly and wide impacts), empower marginalized people and groups to strengthen their own decision making and research capacity to make effective demands on research and extension services and thus have payoffs both for farmers and for scientists.

Exploiting Multiple Benefits of Legumes

Legumes very often provide other benefits besides fixed N to the cropping system of which they are part. Although rotational effects of legumes on subsequent cereals have often been translated into N fertilizer replacement values, rotational benefits cannot always be explained in terms of N addition to the system. Besides improving the soil physical structure, deep-rooting perennial species may recover nutrients from the subsoil and reverse topsoil degradation (e.g., reverse soil acidification caused by fertilizer use, Vanlauwe et al. 2001). Legumes have also been shown to alter pest and disease spectra and to reduce Striga incidence. All the above processes are alleviating a constraint to crop growth and may consequently lead to improved use efficiency of applied N fertilizer, following the Indirect hypothesis.

Development Needs

Innovations can be considered as demand-driven or supply-driven. It is fair to say that in the eyes of farmers BNF options may belong to the second category, or at best, are a mixture of both. Furthermore, soil fertility decline as an ISFM issue is complex, difficult to prevent given farmers' situations, and easy to detect only when yields drop sharply. This infers that many ISFM innovations will be most effective as conservative or preventive innovations; adopting often means sacrificing short-term profits for reducing a decline in returns in the future. These innovations often have slow rates of adoption. Simultaneously, farmers vary in their risk preferences of an innovation, and perceptions are affected by information introducing further heterogeneity due to different sources of and exposure to information. Often farmers do not face the problem targeted by the innovation, or the innovation simply does not work. In addition, farmers will not commit to adoption of an innovation without successfully trying it. If small-scale trials are not possible or not enlightening for some reason, as is frequently the case in heterogeneous and fragile environments that are target regions for BNF, the chances of widespread adoption are greatly diminished. Conducting a trial incurs costs of time, energy, finance and land that could be used productively for other

purposes. Furthermore, the fact that economic and environmental conditions are rapidly changing today makes the adaptation of present land use systems and the process of including BNF in ISFM largely a process of managing the uncertain.

By taking a pro-poor approach, international agricultural research has developed the means to achieve large-scale impacts, responding to the demands of small-scale farmers for improved agricultural production and ecosystem services. Many ISFM options are locally profitable, even under intensely cultivated land-scarce conditions. The knowledge-intensity and complexity of the ISFM approach, however, makes it difficult to translate local successes from one area to another, unless the factors favoring and constraining adoption are better understood. Increasing our understanding of where ISFM options are working, why, and for whom, will address the constraints limiting their wider use. The cost of not engaging in this research is likely to be enormous, in terms of greater poverty, stagnant and declining production, degraded ecosystem services, and the loss of intellectual property rights related to the local genetic resources of the soil.

Facilitating widespread use and impact of ISFM to solve soil fertility problems in the tropics will thus require a tighter linkage and feedback between strategic and adaptive research activities. The iterative process of learning and problem solving builds on indigenous knowledge, improves imperfect technologies, and empowers farmers and institutions. Addressing farmers' problems in a systems context generates management options better suited to their local needs. It also produces policy options that are suited to local institutional realities.

Involving Stakeholders in the Technology Development Process

The model of involving farmers in research is based on strong evidence (Pretty and Hine 2001) that enhancing farmers' technical skills and research capabilities, and involving them as decision-makers in the technology development process, results in innovations that are more responsive to their priorities, needs and constraints. It is now widely recognized that these FPR approaches may have wider applications for improving rural livelihoods in complex and diverse low potential areas where a 'systems' approach is critical for the analysis and improvement of the production systems (Okali et al. 1994).

The active involvement of producers in the design of the ISFM system enables researchers and stakeholders to examine and understand the local farming systems and the larger context within which they exist, to incorporate local knowledge into technology innovation, and to develop locally appropriate solutions. A hallmark of the FPR approach is the link it establishes between the formal and local research systems (Ashby et al. 2000). This link

enables farmers to express their technology needs and to help shape the technology developed through formal research. Participatory research decentralizes control over the research agenda and permits much broader set of stakeholders to become involved in research, thereby addressing the differential needs of men and women for technical innovation.

Finally, farmer participatory experimentation and learning approaches represent an investment in the human and social capital available to poor farming families that can be harnessed to provide a systematic feedback process on farmers demands and priorities to research providers. These approaches build farmers' capacity to learn about knowledge intensive processes, and biological and ecological complexities (Pretty and Hine 2001) and can create a sustained, collective capacity for innovation focused on improving livelihoods and the management of natural resources.

Identification of Uncertainty Within a Cropping Systems Approach

Scientific and local knowledge can be analyzed in relation to prevailing uncertainties about the innovation using an approach to uncertainty suggested by Rowe (1994). Rowe explains how uncertainty extends through many parts of the decision problem by distinguishing temporal, metrical, structural and translational uncertainty. Temporal uncertainty is associated with fluctuations of processes over time. Metrical uncertainty is introduced by errors associated with the estimation of parameters in a spatially varying resource base. Structural uncertainty is related to the imperfection of the decision model itself. Translational uncertainty arises from contrasts between the perspectives of individuals involved in the decision process.

For example, in deciding how to apply fertilizer, a more precise definition of the relationship between inputs and response could reduce metrical uncertainty. Unlike farmers in highly intensive cropping systems, small-scale farmers in tropical systems do not have ready access to modern monitoring techniques. But they do possess a long time series understanding of relations at one location, which has been generated through repeated observations. These accumulated observations can be related to relevant scientific soil parameters presented above, or their local counterparts, providing opportunities for the development of spatially explicit indicators.

Temporal uncertainty could be reduced by specifying the phase of crop development for which such a relationship is valid. Farmers have already assembled plenty of experience doing this when deciding, for example, when to enter the productive system. Scientists can help to render farmers' experiences in traditional systems transferable to new cropping circumstances by relating them to underlying processes. On this basis, for example, indicator plants can specifically be selected and grown in new cropping systems. Simple monitoring devices such as leaf color meters provide further opportunities.

Structural uncertainty could be reduced by defining more of the interactions of fertilizer applications with other variables, such as pest and weed infestation or rainfall. Translational uncertainty could be reduced by formulating the actions suggested to reduce the other types of uncertainty in terms relevant to the hillside farmers. Reducing structural and translational uncertainty is probably least amenable to formal scientific investigation – structural uncertainty because of the huge complexity of the interactions and the variation in the natural resource base in hillside environments, and translational uncertainty because of the little attention given by scientists to what really matters to farmers. To reduce the former, scientists need to understand whether variation matters to farmers, and if so how much of it farmers are willing and able to manage. Relevant and informative trials are essential.

Identification of Niches Within a Cropping Systems Approach

If farmers had complete information, innovations recognized as relevant would be implemented immediately. Information about complex farming systems and their externalities is however not complete. A pragmatic choice of whether or not to implement an innovation at farm level has to be made about whether or not it is sensible to manage variation more closely. This is based on the interrelated questions of whether as-yet unmanaged variation is significant, whether it is controllable and predictable. All three conditions of significance, control and predictability must be satisfied before improvement can occur.

Significance: this is largely a question to be decided by individual farmers. But research has demonstrated that farmers are well aware of problems, and their natural tendency to experiment demonstrates their willingness to change.

Control and prediction: in most farms, there is uncontrolled variation that is usually of no benefit to farmers. Farmers have the capacity for field-by-field control, and some in-field control. However, this is limited by farmers' experiences based on long-term observations that usually do relate to traditional cropping systems and control by these means cannot directly be used for new innovations. Second, for control to be effective, the relationship between variation of the controllable inputs and output must also be known to some degree.

The key to reducing uncertainty is on-farm trials, preferably on the farmer's own property. For these reasons, rapid adoption of ISFM management options, involving combinations of unfamiliar and complex innovations that are difficult to test, are unlikely to occur until farmers consider them relevant and essential. Furthermore, even if they are considered relevant and essential, appropriate designs of trials have to be defined that overcome obstacles, and take into consideration the following facts:

- Treatments often must be implemented in combinations that make it difficult to determine from field observations alone the individual impacts of each element of the combination. For a trial to be worthwhile, the results of the trial must be observable.
- The effectiveness of some innovations may be very sensitive to temporal changes (e.g. weather conditions) or the quality of implementation. Therefore, trials give highly variable results from time to time.
- Economic comparisons based on typical agronomic small-scale research trials can be very misleading. However, the larger the trial is, the less likely the farmer is to invest in trials.

Improving Adoption and Impacts of ISFM Approaches

Principles of ISFM could influence stakeholders in the tropics to alter the way they address soils and their management, at a variety of levels. Promotion of ISFM approaches will require increasing participation of national and international research and development organizations, networks, NGOs, and extension agencies working in the tropics. Significant adoption of a range of ISFM technologies has been documented across a number of countries in sub-Saharan Africa. These include (1) integrated nutrient management, (2) micro-dose use of fertilizers, (3) improved manure management practices, (4) intercropping systems, (5) integration of multipurpose legumes, (6) improved fallows, and (7) biomass transfer of high quality organic inputs. However, most of these adoption studies have focused on conventional factors influencing adoption of agricultural technologies. The complexity of ISFM requires the identification of farmers' decision-making processes, constraints and opportunities for the adoption of ISFM technologies, and the identification of farmers' criteria for acceptability of BNF technologies. For this, the complex linkages between livelihood assets and strategies and ISFM adoption, and the impacts of ISFM technologies on rural livelihoods must be better understood. Measuring the impacts of ISFM is a complex task. We need to develop innovative methods that enable us to track changes in the systems using participatory monitoring and evaluation systems to learn from successes and failures.

Building Capacity at Different Scales

The capacity for ISFM research in the tropics is insufficient both in terms of the numbers of professional personnel and the essential laboratory facilities. ISFM is a knowledge-intensive approach to soil management. Professional staff and students alike suffer from isolation and lack of access to up-to-date educational opportunities. Networks run by SROs and CGIAR Centers, such

as the TSBF African Network for Soil Biology and Fertility (AfNet) and MIS (Integrated Management of Soils) consortium in Central America provide a vehicle of opportunity to correct this situation. A substantial number of short-term, degree-related, and on-the-job training activities across the tropics could help spread ISFM approaches at all national levels, including university curricula.

Some of the groundwork for scaling up and out has been laid through an emphasis on the synthesis of results and dissemination of information on the technologies and on developing partnerships between research, extension services and NGOs. TSBF-CIAT researchers have experience in developing and applying decision guides to assist extension staff and farmers in selecting among soil fertility options for different situations (Palm et al. 2001). The use of accessible, user-friendly GIS tools and geo-spatial datasets for the region can be used in the scaling process, by identifying recommendation areas for BNF technologies.

Scaling up requires sustained capacity building to build the requisite skills among the NARS to ensure that the work is involving and reaching the intended beneficiaries. It also requires building local capacities and empowering rural communities to improve their technical skills and decision-making on soil fertility, in support of scaling up and sustaining impacts of ISFM technologies. Efforts to engage with policy makers and private sector input suppliers and dealers should also be strengthened.

SUMMARY AND CONCLUSIONS

In this position paper, we have argued that BNF is a key input to ISFM strategy to combat soil fertility degradation and for sustainable intensified agriculture in the tropics. The reasons for lack of success in solving the soil fertility problem lie substantially in the failure to deal with the issue in a sufficiently holistic way. Soil fertility decline is not a simple problem. In ecological parlance it is a 'slow variable', which interacts pervasively over time with a wide range of other biological and socioeconomic constraints to sustainable agroecosystem management. It is not just a problem of nutrient deficiency but also of inappropriate germplasm and cropping system design, of interactions with pests and diseases, of the linkage between poverty and land degradation, of often-perverse national and global policies with respect to incentives, and of institutional failures. Tackling soil fertility issues thus requires a long-term perspective and holistic multidisciplinary systems approach of ISFM.

Developing adoptable legume-BNF technologies to combat soil fertility degradation remains a major challenge. Research and development efforts are needed to integrate BNF efficient and stress-adapted grain and multipurpose legume germplasm into production systems to intensify food

and feed systems of the tropics. Several key interventions are needed to achieve greater impact of legume-BNF technologies to improve livelihoods of rural poor. These include (a) integration of stress-adapted and BNF-efficient legume cultivars in rotational and mixed cropping systems, (b) strategic application of inorganic fertilizers and organic residues to facilitate efficient nutrient cycling and appropriate replenishment of soil organic matter, (c) adoptable strategies of soil and water conservation, (d) integrated pest/disease/weed management through the use of biotic stress-resistant germplasm with minimum pesticide/herbicide applications, (e) marketing strategies that are economically efficient, and (f) development of an appropriate policy and institutional environment that provides incentives to farmers to adopt legume-based BNF technologies.

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7

The Ability to Fix N is not the Only Key to Delivery of the Benefits of BNF to Farmers: Experience of IITA in the Savannas of Africa

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ABSTRACT

The IITA in its research for sustainable soil fertility management has passed through several stages during which legume-based systems were always at the forefront. Alley cropping has N yields of more than 200 kg ha^{-1} with 50% N_2 fixation. In the case of *Mucuna* cover cropping, the ecological benefit was unequivocal, with more than 100 kg ha^{-1} of N_2 fixed and consistently positive effects on crop yields. But adoption by farmers has been too low to bring the benefit of BNF to farm households. Based on these experiences, we have proposed increased emphasis on systems based on cowpea and soybean rotation because of their high adoptability although the benefits to the soil are small compared with *Mucuna* fallows and alley farming. Besides high protein grain production, cowpea and soybean rotation reduced densities of *Striga hermonthica* by 50% in farmer-managed trials and increased net benefits to farmers. Cover cropping and agroforestry systems can also be used for soil fertility management, but only if their adoptability increases by providing products needed by farmers.

INTRODUCTION

IITA has pursued the development of low-input sustainable agriculture since the mid-1970s. Planted leguminous fallows were developed as an alternative

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to N fertilizer, as sources of organic matter, and techniques for weed suppression. The technology development was initially dominated by agroforestry and cover cropping. More recently the team working in the savanna zone has pursued much more aggressively the development of grain legume rotation systems with a view to improving their benefits to the resource base. The systems will be examined first for their biological benefits and subsequently for their adoptability. The lessons learned from adoption studies of these systems are synthesized to guide future research for development.

BIOLOGICAL ATTRIBUTES OF LEGUME-BASED SOIL MANAGEMENT SYSTEMS

Alley Cropping

The agroforestry system that was developed to the greatest extent was called alley cropping and consisted of trees grown in rows planted approximately 4 m apart with food crops in the *alleys* between the rows of trees. The term *alley farming* was used when fodder was harvested from the trees. In addition to fodder, other products that are possible, if appropriate woody species are used, include fuel wood, construction material and medicines. Kang and Shannon (2001) reviewed the literature on alley cropping and found that N yields are typically around 200 to 300 kg ha^{-1}. Of this, approximately 50% of the N is derived from the atmosphere (Giller 2001). Typical N contributions to the subsequent crop of maize compared to direct N fertilizer applications (N fertilizer replacement value) have been approximately 45 to 90 kg N ha^{-1} for alley cropping with *Gliricidia sepium* or *Leucaena leucocephala* in the humid zone of southwestern Nigeria (Atta-Krah and Kang 1993, Sanginga et al. 1986, Tian et al. 1993). Substantially lower N contributions also occur, especially when the system is established in drought-prone areas or on acid or infertile soils. Generally, soil fertility is maintained as soil erosion is reduced and organic matter levels are maintained (Kang and Shannon 2001). However, crop yields may not reflect the soil fertility increase because of the reduction in planted area and because of competition between trees and crops for water and nutrients. Versteeg et al. (1998) recorded the results of farmer-managed trials in southern Benin in which maize yields in alley cropping were lower than the control without trees in four out of five years.

Cover Cropping

The most popular cover cropping systems have been based on *Mucuna pruriens* var. *utilis* accession 'Utilis' or 'Cochinchinensis' (or simply 'mucuna') in annual crops and *Pueraria phaseoloides* in humid zone plantations (oil palm and rubber). Mucuna has been used extensively during the early 1900s

in the southeastern USA (Eilittä and Sollenburger 2002) and Zimbabwe (Maasdorp et al. 2002). Recently, research and development organizations have proposed one-year mucuna fallows to farmers in the tropics. A recent synthesis of literature showed that mucuna usually fixes more than 100 kg N ha^{-1} in humid and subhumid moisture regimes unless soil P is deficient or the soil is acid (Carsky et al. 2001, Giller 2001). Analysis of 16 trials in the tropics suggests that maize yields after one year of mucuna fallow are increased by 1.0 t ha^{-1} compared to a natural (weedy or grass) fallow control and by 1.6 t ha^{-1} compared to a continuous maize control (Carsky et al. 2001). The same dataset suggests that the maize grain yield increase is 1.8 t ha^{-1} when the mucuna residues are incorporated and 0.8 t ha^{-1} when left on the soil surface. A mucuna fallow that accumulates 4-6 t ha^{-1} of dry matter can be expected to replace 30-80 kg ha^{-1} of N fertilizer (Carsky et al. 2001) when left on the soil surface as mulch.

An additional benefit of mucuna is weed suppression. Mucuna has large seeds approaching 1 g in weight. Thus, early growth is rapid and cover is usually complete in 6 to 8 weeks. In West Africa, one of the most common indicators of degraded vegetation is *Imperata cylindrica* or speargrass. Because this weed propagates from rhizomes, it is not possible to eradicate, even with intensive weeding by hand. It is therefore a major cause of abandonment of food crop production on infested land. In a trial in southern Benin, Chikoye et al. (2002) observed that a two-year mucuna fallow nearly eliminated speargrass rhizomes. However, the ability to suppress speargrass varies with mucuna accessions, of which there are many (Chikoye and Ekeleme 2001).

Soybean Rotation

Soybean can be expected to fix approximately100 kg N ha^{-1} under suitable savanna conditions (Sanginga et al. 2001, Singh et al. 2003) but less than 50 kg ha^{-1} in infertile or acid soils (Singh et al. 2003). The N contribution of soybean to the soil-plant system is much lower than that of leguminous trees and cover crops. An estimate of the direct contribution of soybean to maize was obtained by Sanginga et al. (2001) by the indirect ^{15}N labeling method. Residual N values between 10 and 24 kg ha^{-1} were obtained in the first maize crop after soybean, representing 14% to 36% of the maize total N, while the total N difference method gave values varying between 16 and 23 kg N ha^{-1} (Sanginga et al. 2001). It is clear that the BNF benefit to nonlegumes due to the inclusion of legumes in a cropping system is small indeed compared to the level of N needed for high yields (Carsky and Iwuafor 1999). Published estimates of N fertilizer replacement values (NFRV) from soybean in the mono-modal savanna zone of West Africa range from 20 kg N ha^{-1} (Carsky et al. 1997) to 45 kg N ha^{-1} (Kaleem 1993). These estimates appear to be high in

comparison with recently completed trials in several sites in northern and central Nigeria with several soybean lines. Ogoke et al. (2001) reported NFRV of 0-17 kg ha^{-1} and Singh et al. (2001) found mean values of approximately 5, 10 and 20 kg ha^{-1} when above-ground soybean residues were exported, surface-applied, and incorporated, respectively. However, NFRV reflects not only the direct effect of N_2 fixation but also the N-sparing effect. The benefits of bringing the grain legume into cereal monocropping might be higher than are expected from the net contribution from the legume because the alternative (a cereal crop) depletes soil N. Also there are non-N benefits of soybean rotation. For example, Carsky et al. (2000) reported reduced *S. hermonthica* (witchweed) parasitism on maize after soybean compared with a sorghum control.

Cowpea Rotation

A review of several trials in the savannas of West Africa (Carsky et al. 2002) suggests that cowpea can be expected to fix between 50 and 100 kg N ha^{-1}. Cowpea tends to accumulate a smaller fraction of its N in the grain than soybean; therefore, it can be expected to have a slightly larger effect on subsequent cereal yields. The estimates of N fertilizer replacement value for cowpea range from 10 kg N ha^{-1} (Carsky et al. 1999) to 80 kg N ha^{-1} (Horst and Hardter 1994). Higher estimates were observed where residues were incorporated into the soil (Kaleem 1993, Dakora et al. 1987) or where two crops of legume were grown in one season (Horst and Hardter 1994). NFRV was only 9 kg ha^{-1} for cowpea in the first year followed by maize in the second year in the Guinea savanna of northern Nigeria at latitude 11°N (Carsky et al. 1999). This suggests that a long dry season reduces the benefit of cowpea. Jeranyama et al. (2000) grew cowpea and crotalaria as relay intercrops with maize for two years followed by a maize test crop in the third year and calculated a NFRV of 36 kg ha^{-1}. Bagayoko et al. (1997) estimated the NFRV to be approximately 40 kg ha^{-1} at Cinzana, Mali (latitude 13° N). Thus, a part of the N requirement of cereal crops can be satisfied by cowpea crop rotation (Carsky et al. 2002).

Cowpea rotation benefits cereals in ways similar to soybean. The NFRV is a reflection of direct N contribution plus the N-sparing effect. Non-N effects are rarely understood. The results of Reddy et al. (1994) suggest that the effect of cowpea rotation appeared to be related to incidence of *S. hermonthica* on the cereal test crop as there was more *S. hermonthica* on millet after millet than on millet after cowpea. It is not clear whether cowpea actually reduced *S. hermonthica* incidence or whether it simply did not result in build-up as the millet did. Ariga et al. (1994) observed that a preceding crop of cowpea variety TVx 3236 reduced *S. hermonthica* density on a subsequent maize crop and increased maize yield. The effect increased with the duration of growth of

the cowpea crop. Generally, non-N benefits may be estimated by contrasting the benefits of rotation with a monoculture control (e.g. millet after millet or maize after maize) with a non-monoculture control (e.g. millet after sorghum). Yield increase after cowpea compared with monoculture cereal was 80%, but only 31% when the control was not a monoculture. A monoculture control treatment may have more pest and disease pressure than a non-monoculture cereal rotation system and many non-leguminous crops could provide the same benefit as cowpea (providing a break in pest and disease cycles).

LESSONS LEARNED FROM PARTICIPATORY RESEARCH WITH PLANTED FALLOWS

Alley Cropping

Douthwaite et al. (2002) describe the history of alley cropping with some benchmark events. The first major event was the publication by Kang et al. (1981) that the technology could maintain maize yields after 4 years of cropping while control yields declined. The Alley Farming Network for Tropical Africa (AFNETA) was formed in 1989 in recognition that the technology should be tested in many areas. By 1992 there were AFNETA trials in 20 countries and many publications on alley cropping.

Adoption studies by Whittome (1994), Dvorak (1996) and Swinkels and Franzel (1997) show limited adoption potential for alley cropping. On-farm experience suggested to Dvorak (1996) that "productivity gains might be sufficient to induce adoption of alley cropping on farms planted with sole maize on soils whose baseline yields are below 2 t ha^{-1}, yet are still good enough to respond to application of nitrogen, and whose farmers have a flexible command of labor during time of pruning". Whittome et al. (1995) proposed targeting adoption to areas where (1) maize is the dominant crop, (2) annual rainfall is sufficient to avoid competition between crops and trees, (3) land is scarce (engendering scarcities of wood and diminishing soil fertility), and (4) individual ownership is common. A map of the zone where maize is a suitable crop, rainfall exceeds 1200 mm yr^{-1}, soils are not acid, and population density exceeds 30 persons km^{-2}, left huge areas where alley cropping should not be proposed. It was suggested by Douthwaite et al. (2002) that there was little farmer-to-farmer diffusion as the data of Adesina et al. (1997) suggests that farmers did not test alley cropping if they learned about it from other farmers (Table 1).

Meanwhile, other agroforestry systems are adopted in West Africa. The traditional agroforestry system in southern Benin is an oil palm fallow (Kang et al. 1991). While improving the soil, it produces some oil and weaving material and after the fallow it produces palm wine and whiskey. These products all serve to generate cash and make the oil palm fallow an investment

Table 1. Testing of alley farming (number of farmers who tested/number of farmers informed) as influenced by the source of information.

Source of information	Nigeria	Benin
Researcher	134/164	46/125
Extension	0/3	26/74
Farmer	4/40	0/24

Source: Adesina et al. (1997)

with direct economic benefits. Another agroforestry system currently gaining in popularity in southern Benin is the *Acacia auriculiformis* woodlot. The major product of the system besides soil fertility improvement is wood for fuel, construction and furniture, rare commodities in the overpopulated savanna zone near the coast of Benin. In some parts of southern Benin, projects are still involved and subsidizing the system, but in other areas it has continued to expand even after projects pulled out (Douthwaite, Carsky and Floquet, unpublished 2001).

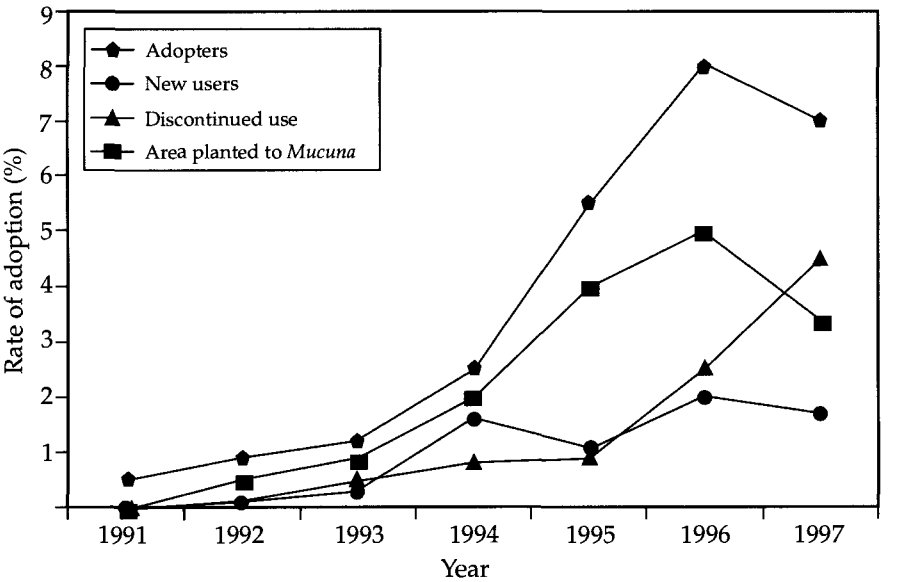
The lesson learned from alley cropping is that direct economic benefits are needed for adoption to occur, not just soil improvement. In the case of agroforestry in high population density zones, the most likely economic product is wood but others (fruit, medicines, animal feed, etc.) are possible. If wood is most likely to be the primary direct economic output of alley cropping, it must be targeted to high population density areas where wood is needed. We also suspect that adoption may be favored if fertilizer is not easily available, but this is not proven.

Cover Cropping with Mucuna

Mucuna has had a relatively long history in Nigeria and it was one of many cover legumes being tested in live-mulch systems at IITA in the mid-1980s (Akobundu 1992). Douthwaite et al. (2002) summarized the history of mucuna fallow technology generation. Mucuna was tested in a participatory mode with several other technologies for soil fertility maintenance in southern Benin in the late 1980s. Some farmers observed that a mucuna fallow weakened speargrass and made it easier to control. There were subsequently many more requests to research and development agencies for seed. Initial adoption was reported by Manyong et al. (1996) to be relatively high in villages where it was initially tested. The fact that farmers reacted more to weed suppression by mucuna indicated again that the soil fertility benefit alone is not sufficient to promote adoption of improved fallows that take land out of production. The ability to weaken speargrass was a major advantage that promoted adoption.

In Benin mucuna seed was generally given to farmers for trials and, because mucuna fallow was an expanding technology, there was often an

artificial market for mucuna seed. Manyong et al. (1996) calculated the temporal trend of benefit/cost for an adopting farmer who uses mucuna and found that sale of mucuna seed doubled the benefit-cost ratio. This showed the importance of a direct economic product from the fallow system as mentioned above for alley cropping. A subsequent survey in southern Benin after the big push by development projects (Honlonkou et al. 1999) showed that adoption rates were actually decreasing and abandonment was increasing (Fig. 1), probably because the market for mucuna seed was decreasing. There have been no subsequent adoption studies but informal discussions with farmers indicate the lack of market for seed as important in their decision to abandon mucuna.



Source: Honlonkou et al. (1999).

Figure 1. Dynamics of mucuna fallow adoption in southern Benin from 1991 to 1997.

In many systems the niche occupied by mucuna takes land out of production for a food crop. In a comparison of grain legumes with mucuna in Kaduna (northern Nigeria), maize yields after mucuna were higher than after cowpea but the latter system was more economically beneficial (Oyewole et al. 2000). Farmers in the study site eventually abandoned mucuna in favor of cowpea.

These experiences led Carsky et al. (2001) to predict relatively limited adoption in West Africa based on the known benefits of soil improvement and weed suppression. This is mostly due to the fact that mucuna occupies the land without a direct economic product. However, other possible benefits

of mucuna may exist. For example the use of seed or foliage of mucuna in human or animal nutrition is being tested. Research teams have recently shown the value of inclusion of seed and pods (Castillo-Caamal et al. 2003, Mendoza-Castillo et al. 2003) or leaves (Muinga et al. 2003) in diets of small ruminants. Additional benefits to the farm household would increase adoptability beyond the zone predicted by Carsky et al. (2001). Other opportunities are special niches related to globalization. For example, mucuna fallow may be found to be the best way to supply N and suppress weeds for organic cotton in Africa for the European market.

As with alley cropping, it became clear that soil fertility improvement is not enough to drive the system in West Africa. In Benin, the problem of speargrass infestation was more clearly perceived than poor soil fertility. Thus, legumes with multiple benefits are more likely to be adopted and legume benefits that are solutions to problems perceived by farmers help promote adoption. Yet, resource-poor farmers need a direct economic benefit and that appears to be why the use of mucuna is no longer increasing.

LESSONS LEARNED FROM A FOCUS ON GRAIN LEGUME ROTATIONS

While IITA and partners were struggling to find niches for alley cropping and mucuna fallow, cowpea was already being extensively used in West Africa (Schulz et al. 2001) with 6 to 9 million ha of land area in West Africa from 1961 to 1990. Also, soybean crop area was increasing, especially in Nigeria. According to FAOSTAT (2002) soybeans were planted on 742,000 ha in West Africa in 1990, 98% of which was in Nigeria.

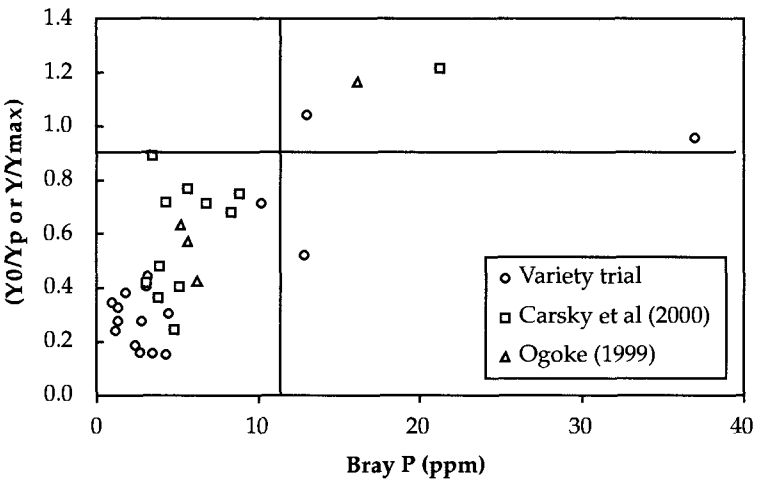
In Nigeria, IITA soybean varieties were adopted in the late 1980s and early 1990s through the efforts of nongovernmental and government development organizations. In Benue State, a soybean-growing area for several decades, Sanginga (1998) observed that more than 50% of randomly selected farmers had adopted new IITA varieties during a 10-year period. In nearby southern Kaduna State, a development organization gave seed of improved soybean to farmers. Manyong et al. (1998) found that by the third year, 35 farmers had passed seed of the new varieties to 45 additional farmers. These varieties were resistant to shattering of pods before harvest, had good seed viability and good resistance to pests and diseases.

Adoption of soybean in Nigeria was further stimulated by a large effort to develop food recipes using soybean, and incorporating soybean into traditional Nigerian dishes (Osho and Dashiell 1998). There has been a big increase in the demand for soybean in most of the major cities in Nigeria. An example of this is Ibadan (one of the largest cities in Nigeria), where an urban market survey revealed that soybean was sold in only two markets in

1987 and 19 markets by 1990. Soybean retailers in those markets expanded from a total of 4 to 419 during the same period (Osho and Dashiell 1998).

With this in mind, IITA changed focus in the mid-1990s, with an emphasis on grain legumes, especially those that are being bred at IITA. This was done realizing that the potential benefit of grain legumes to the soil is relatively low but that these technologies are infinitely more adoptable than other legume-based technologies. Grain legume rotations began to be thought of as 'planted fallow' systems with a direct economic product. One of the additional benefits of soybean and cowpea rotation was that they could be false hosts for *S. hermonthica*. This was first discovered by screening a large number of soybean lines using an *in vitro* technique and subsequently validating the results in the field (Berner et al. 1996). This gave grain legumes another potential benefit in addition to grain for human nutrition and soil improvement.

Thereafter substantial resources in IITA have been put into the grain legume-cereal rotation system, especially for soil fertility improvement and *S. hermonthica* reduction. From biological research focused on soybean in the rotation system, we have developed recommendations related to the need for inoculation, the need for P fertilizer, and the maturity cycle of the legume. IITA soybeans only responded to inoculation with introduced rhizobia when the density of indigenous rhizobia was lower than 10 cells g^{-1} soil (Sanginga et al. 1996). The critical available P level (the concentration above which fertilizer amendments are not needed) for soybean is approximately 10 $\mu g\ g^{-1}$ (Fig. 2).



Y0/Yp is yield without P fertilizer divided by yield with P fertilizer. Y/Ymax is yield of soybean divided by the highest yield in a multi-site variety trial without P applied.

Figure 2. Critical plant available P level for soybean in the savanna zone of Nigeria from Carsky (unpublished data from variety trials conducted in 1997); Carsky et al. (2000); Ogoke (1999).

Table 2. N balance (kg ha^{-1}) of soybean crops (with haulms returned) in multi-locational trials (3 or 4 sites per trial) in northern Nigeria.

Soybean line	Maturity	Trial 1	Trial 2	Trial 3
TGx 1485-1D	early	-5.4	-7.2	-12.2
TGx 1805-2E	early	-3.9	—	—
TGx 1681-3F	early	-7.5	—	—
TGx 536-2D	medium	—	—	-2.6
TGx 1809-12E	medium	+1.7	—	—
TGx 923-2E	late	+9.5	—	+1.6
TGx 1670-1F	late	+1.5	+3.8	+10.9

Source: Trial 1 = Singh et al. (2003), Trial 2 = Singh et al. (2003), Trial 3 = Ogoke et al. (2003)

Finally, in choosing a grain legume variety for a particular agroecological zone and cropping system, the latest possible maturity cycle will give the maximum N balance and residual effect (Table 2). In addition, genetic improvement has focused on legume traits that will improve the benefits of grain legumes to the farming system (biological N_2 fixation, P use efficiency, fodder production, and *S. hermonthica* seed germination). At the same time maize germplasm was developed for *S. hermonthica* resistance and N use efficiency.

The most important lesson learned is that the market dictates in great measure what constitutes appropriate technology. Therefore biological scientists must be aware of the need for identification of markets for the commodity or efforts to promote markets for the commodity. For the cereal-based system in the savanna zone, the ability to stimulate suicidal germination of *S. hermonthica* appears to be a key criterion for adoption of grain legume varieties. In addition, they should be resistant to parasitic weeds (*Striga gesnerioides* and *Alectra vogelii*) and provide fodder.

THE WAY FORWARD FOR THE GRAIN LEGUME ROTATION SYSTEM

Although the soil fertility benefit of soybean or cowpea is low to moderate, farmers benefit from the production of pulses for sale or consumption, reduced *S. hermonthica* where it is a problem, and some fodder production where needed. Thus, soybean and cowpea rotations with maize were tested intensively with farmers in northern Nigeria, starting in 1998. Testing the grain legume rotation systems poses little risk to farmers compared to alley cropping and cover cropping. The latter systems require substantial investment in labor for eventual increase in yield. If abiotic or biotic stress unrelated to soil fertility occur, then maize yields do not compensate the investment.

Effects of grain legume rotation on *S. hermonthica* parasitism under farmer management are as favorable as in previous researcher-managed studies. In

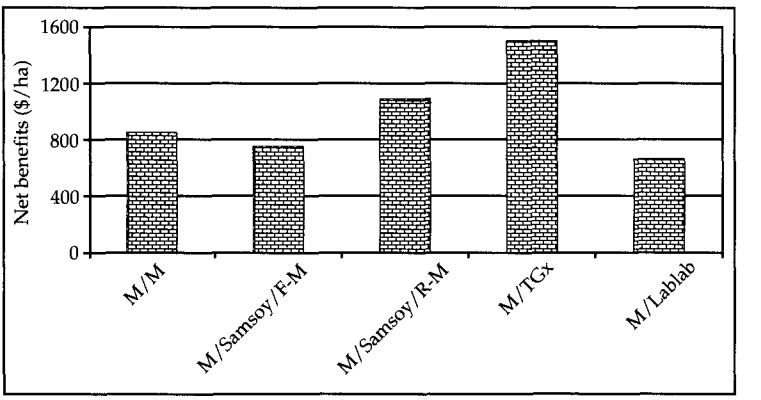
one study conducted in several villages in northern Nigeria by Schulz et al. (2003), soybean and cowpea were used to reduce *S. hermonthica* seed density in the soil. After one year of soybean (14 fields) or cowpea (5 fields), *S. hermonthica* seed density was significantly reduced from 30,000 to 15,000 seeds m^{-2} (Table 3). *S. hermonthica*-resistant maize was grown after soybean or cowpea rotation and compared with the farmers' current variety. Subsequent density of emerged *S. hermonthica* plants on maize was significantly reduced and maize yield was significantly higher in this integrated control package.

Table 3. Initial *S. hermonthica* (numbers m^{-2}) seed densities in the soil prior to (1999) and after one year of soybean or cowpea rotation (2000) on 19 farmers' fields in Kaduna State.

Treatment	1999	2000
Farmer practice	16,594	26,042
Soybean or cowpea rotation	30,081	15,390
Probability	0.8891	0.0560

Source: Schulz et al. 2003

The soil fertility and economic benefit of soybean rotation was also positive under farmer management. In northern Kaduna State, TGx1448-2E fixed 57% of its own N when managed by farmers (Sanginga et al. 2001). Rotation with TGx 1448-2E or Samsoy2 varieties of soybean was compared with rotation with *Lablab purpureus* (lablab) or continuous maize (Sanginga et al. 2003). After TGx 1448-2E, succeeding maize and/or sorghum crops gave good yields with less nitrogen fertilizer than farmers would normally apply. The highest net benefits for the two seasons (1450 US$ ha^{-1}) were obtained with a rotation of TGx 1448-2E followed by maize (Fig. 3). The next



Source: N Sanginga, unpublished.

Figure 3. Net benefit from 2-year rotation of maize (M) followed by maize, Samsoy, TGx1448-2E or lablab at Kaya in northern Nigeria.

best rotation consisting of Samsoy 2 soybean followed by maize gave 1000 US$ ha^{-1}. The lowest net benefits (600 US$ ha^{-1}) were obtained with rotation with lablab or continuous maize (Sanginga et al. 2003). Thus, financial analysis of these systems shows a 50-70% increase in the income of farmers compared to that from continuous maize cultivation. In the subsequent season, all the collaborating farmers opted to grow TGx 1448-2E instead of their own varieties or lablab. Through farmer-to-farmer seed diffusion, more farmers in the villages around the benchmark site had abandoned their old varieties and grew TGx 1448-2E in 2000 (Sanginga et al. 2001). The non-governmental agricultural development organization Sasakawa Global 2000 has started testing the grain legume-maize rotation with large numbers of farmers in northern Nigeria.

A rough estimate of impact assumes an increase in grain legume cultivation area of 10% in the northern Guinea savanna in Nigeria (about 30,000 ha) with yield increases of 20%. This would lead to additional fixed N and P acquisition from sparingly soluble P sources valued annually at $44 million (Sanginga et al. 2003). Production of soybean in Nigeria has been estimated at 436,000 tons in 2001 compared to less than 60,000 tons in 1984 (FAOSTAT 2002). We expect that this estimate will increase dramatically in the next five years.

LIMITS TO GRAIN LEGUME-BASED INTERVENTIONS

While the right technology in the right conditions can make a big difference, we must not forget the importance of the economic environment. Several forces limit the cultivation of grain legumes including low yields (compared with cereals), difficult processing and anti-nutritive factors. It has been found that societies tend to reduce legume production as they develop (Smil 1997). For now, the demand for soybean and cowpea in Africa seem far from saturated but obviously the market is not limitless. The world market price for soybean is much lower than the current domestic Nigerian market price, making the soybean maize rotation system vulnerable and less competitive in the world market. Market information will be crucial in the future to decisions by farmers about what to produce and therefore knowledge of market opportunities is important for research to develop viable food production systems. The payoff from a focus on the right system at local levels is likely to be modified by new rules from international markets, which in the future will be dictating the competitiveness of all production systems at local, domestic, regional and international levels. Therefore, parameters from market globalization must be integrated into the development process of soil management technologies so that production systems remain viable in the long run.

Government policies can have a major influence on what is commercially beneficial to farmers (Keatinge et al. 2001). For example, Adesina and Coulibaly (1998) calculated that the comparative advantage for alley farming in Cameroon increased after the removal of subsidies and the devaluation of the FCFA. Policies could be put in place to favor or disfavor the cultivation of grain legumes and thus all potential scenarios should be studied carefully.

Adequate soil nutrients are essential to the grain legume-maize rotation system because grain legumes do not fix all of the N needed by subsequent maize, and because both crops need other nutrients, especially P. Government policies can influence tremendously the use of fertilizers and sustainable land stewardship in general (Keatinge et al. 2001).

WHO CAN CONTRIBUTE TO DELIVERING BNF BENEFITS TO FARMERS?

Our experience shows that successful delivery of BNF to farmers does not come only from agronomists, breeders, and economists. In the case of the grain legume-maize rotation system, food scientists helped to create a market for soybean, while Striga pathologists helped to identify additional benefits of grain legumes to the cereal systems. Thus, non-traditional disciplines can make a major contribution to delivery of BNF benefits to farmers. A project to deliver BNF to farmers needs to decide how it will encourage other non-traditional disciplines to contribute. For example, animal scientists might define the conditions for use of mucuna grain for animal feed. If farmers use mucuna grain as feed, then the benefit of the mucuna fallow system would become a more realistic means of delivering BNF benefits to them. Similarly, for agroforestry, there may be conditions in which trees can best provide what farmers need. An appropriate agroforestry system can usually be developed to improve the soil as it provides the needed product. But development of knowledge about the product may require expertise completely outside of agriculture.

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8

Enhancement of Symbiotic Nitrogen Fixation in Grain Legumes: Selected Results from the FAO/IAEA Programme

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ABSTRACT

The value of leguminous crops lies in their ability to fix atmospheric N_2, whether grown as pulses for grain, as green manure, as pastures, or as the tree components of agroforestry systems, thereby reducing the use of expensive fertilizer-N and enhancing soil fertility. N_2-fixing legumes provide the basis for developing sustainable farming systems that incorporate integrated nutrient management. The Food and Agriculture Organization of the United Nations and the International Atomic Energy Agency have, through their Joint FAO/IAEA Division in Vienna and the FAO/IAEA Agriculture and Biotechnology Laboratory at Seibersdorf, Austria, coordinated international programs on biological nitrogen fixation (BNF) in developing countries for more than three decades. The main objectives of these programs have been to enhance BNF in various cropping systems. By using ^{15}N, the stable nitrogen isotope, it has been possible to reliably measure rates of N_2 fixation in a wide range of agroecological field situations involving many leguminous species. The accumulated data demonstrate that there is a wealth of genetic diversity among legumes and their rhizobial symbionts, which can be used to enhance N_2 fixation. Practical agronomic and microbiological means to maximise N inputs by legumes have also been identified. Selected results from the FAO/IAEA programme are presented and discussed and references given for further reading.

GRAIN LEGUME CULTIVATION

The area under grain legume cultivation in developing countries has increased slightly over the past three decades (FAO, 2001). The area cultivated to dry bean increased from 20 to 25, groundnuts from 18 to 23, and cowpea

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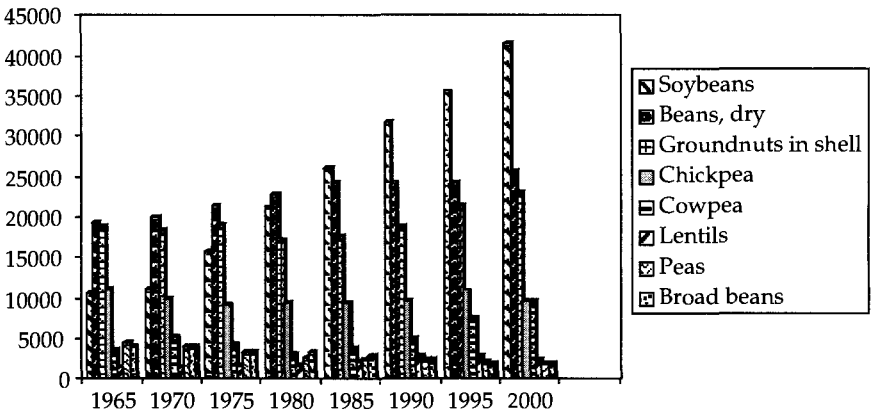
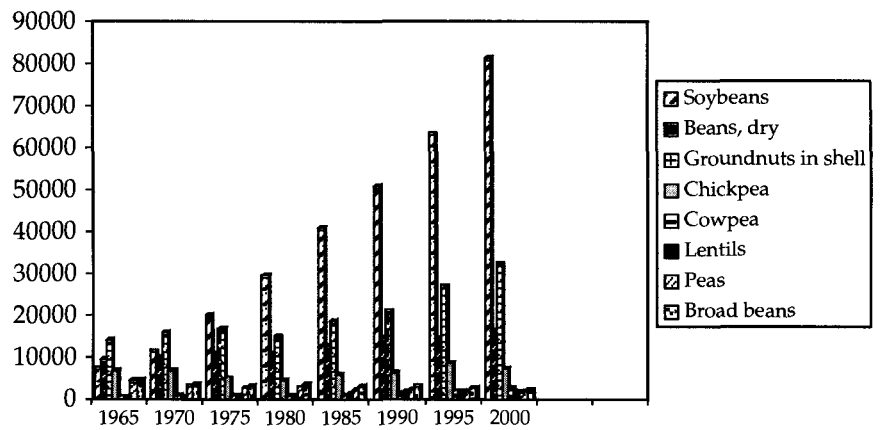
from 4 to 10 million ha (Fig.1). The only major exception was the fourfold increase (from 10 to 40 million ha) in area cultivated to soybean. Other grain legumes, i.e. chickpea, lentils, pea and broad bean have been cultivated on a similar area during this period. The grain legume production shows similar trends with a major increase in soybean production from 8 to 80 million metric tons (Fig. 2). The increase in soybean production took place mainly in Latin America (i.e. Argentina and Brazil), with this crop receiving massive support for improvements such as breeding and inoculant production in the



Source: FAO 2001.

Figure 1. Area (ha x 1000) in developing countries cultivated to grain legumes during the period 1965-2000.



Source: FAO 2001

Figure 2. Annual grain legume production (Mt x 1000) in developing countries during the years 1965 to 2000.

past. Other grain legumes have received much less attention and their production has stagnated as shown in Figs. 1 and 2.

THE VALUE OF FIXED NITROGEN IN MAJOR GRAIN LEGUMES

Based on the area harvested and potential amount of fixed N_2 (Unkovich et al. 2000) the amount fixed by various crops was computed (Table 1). As fertilizer recovery is usually less than 50% under field conditions, the amount of N fertilizer saved was calculated by multiplying the fixation value by two. Assuming the cost of one ton of N fertilizer being US$ 300, then the value of fixed N_2 of soybean and dry bean in developing countries is US$ 3540 and 1300 million, respectively (Table 1). However, it should be considered that the values for the amount fixed per ha might not be accurate and that the cost of one ton of N fertilizer is very variable depending on the type of fertilizer and country. It is therefore clear that N_2 fixed by grain legumes is of great value for agriculture in developing countries.

Table 1. Area planted to various grain legumes in developing countries in the year 2000, the amount of nitrogen fixed and the estimated value of nitrogen fixation in terms of N fertilizer savings.

	Area harvested (million ha)	Fixed N_2* (million MT)	Fertilizer saved (million MT)	Value of N saved (million US$)
Soybean	41.58	5.904	11.80	3,540
Bean	25.61	2.18	4.35	1,300
Groundnuts	23.14	1.16	2.30	690
Chickpeas	9.82	0.56	1.10	330
Cowpeas	9.77	0.49	0.97	290

*Assumed fixation (kg N ha^{-1}): Soybean 142; Bean, 85; Groundnuts 50; Chickpeas 57; Cowpeas 50 (Source:Unkovich et al. 2000)
Source: FAO 2001.

FAO/IAEA PROGRAMS ON BIOLOGICAL NITROGEN FIXATION

The FAO/IAEA has conducted several Coordinated Research Projects (CRPs) on BNF during the past three decades (Table 2). The various CRPs included 7 to 19 participants, mostly scientists from developing countries (Contractors). Cost-free experts (Agreement holders) from developed countries or CG centers assisted in the implementation of the various projects. Experiments conducted as part of the CRPs were performed simultaneously in a number of countries and therefore produced results applicable to a wide range of environmental conditions. Research coordination meetings, funded by the FAO/IAEA, were held to monitor the progress made, to share ideas and responsibilities, to plan future work and to report on results. The nine CRPs mentioned in Table 2 focused on BNF in grain, tree, pasture and forage legumes, *Azolla*, multiple cropping, common bean and microbial ecology. The results have been

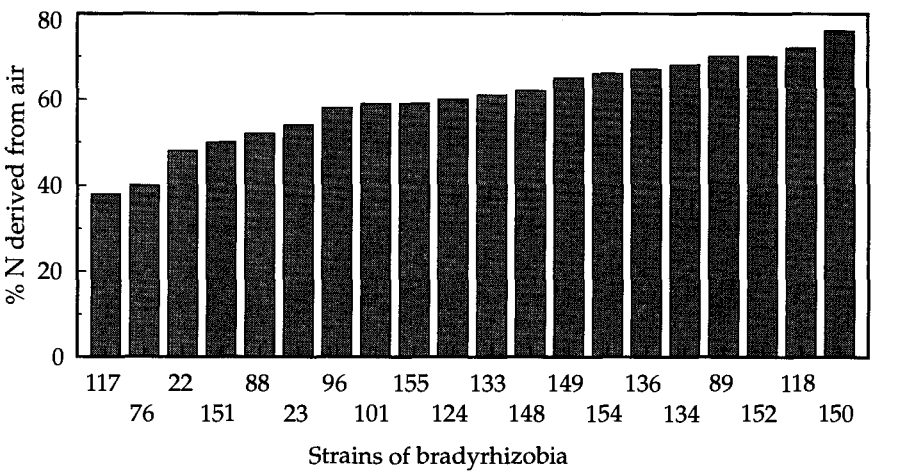
Table 2. FAO/IAEA coordinated research projects on biological nitrogen fixation.

CRPs	Years of implementation	Number of countries participating
Grain legumes I	1972-1977	13
Grain legumes II	1979-1983	19
Multiple cropping	1980-1985	9
Pasture/forage legumes	1983-1988	19
Azolla	1984-1989	13
Common beans in Latin America	1986-1991	7
Grain legumes in Asia	1987-1994	10
Tree legumes	1989-1995	12
Microbial ecology	1992-1997	12

published in several articles (e.g. Bowen and Danso 1987; Hardarson 1994) and books (Bliss and Hardarson 1993; Hardarson and Broughton 1998; IAEA 1983 and 1986; Kumarasinghe and Eskew 1993).

ENHANCEMENT OF SYMBIOTIC NITROGEN FIXATION IN GRAIN LEGUMES

Microbiological, breeding or agronomic methods could be used to enhance symbiotic nitrogen fixation (SNF) (Hardarson 1993). Microbiologists have, in the past focused on enhancing BNF, i.e. selection of rhizobial strains for effectiveness or competitive ability in nodule production for specific legumes. Large variation has been demonstrated between rhizobial strains in effectiveness of N_2 fixation in a field study at the FAO/IAEA Seibersdorf

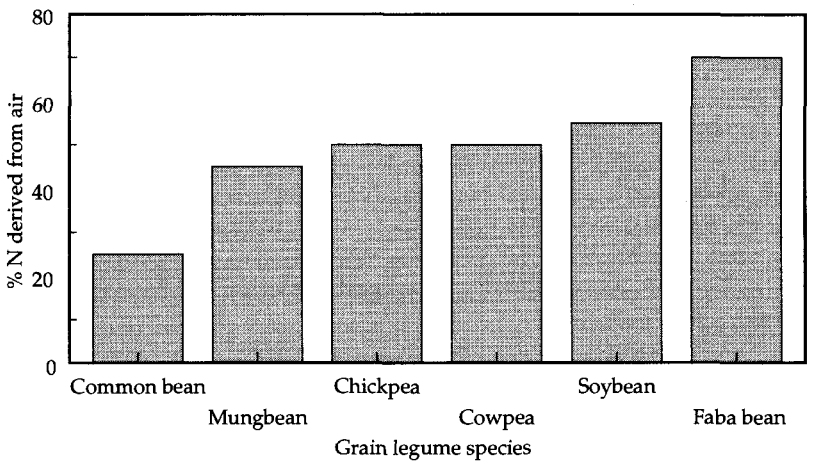


Source: Hardarson et al. 1984.

Figure 3. Variation in the ability of strains of *Bradyrhizobium* to fix atmospheric nitrogen on soybean under field conditions.

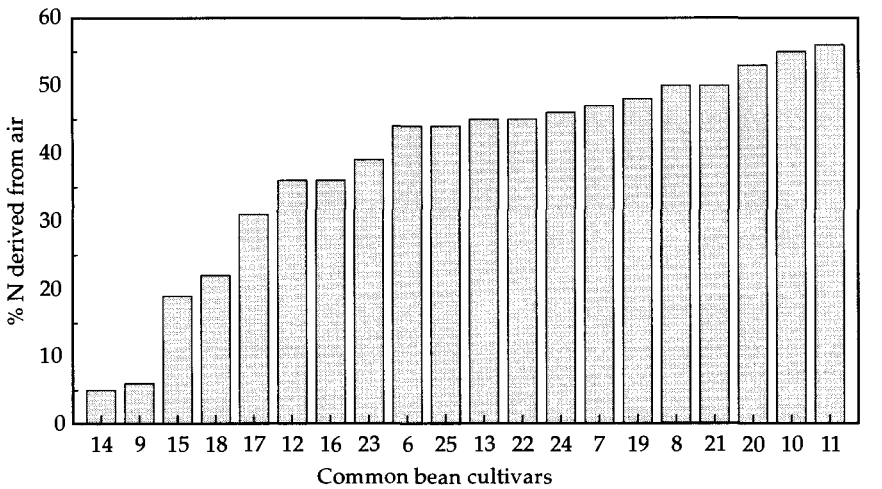
Laboratory (Fig. 3). This is, of course, important when strains are being selected for inoculant production. However, the main problem for microbiologists has always been the competition with indigenous, sometime ineffective, bacteria, and inoculation success has often been limited.

Rhizobial inoculants are being produced in many countries and the availability of inoculants is essential if grain legumes are cultivated on soil



Source: Field experiments in several countries, Danso, personal communication.

Figure 4. Average nitrogen fixation in grain legumes.

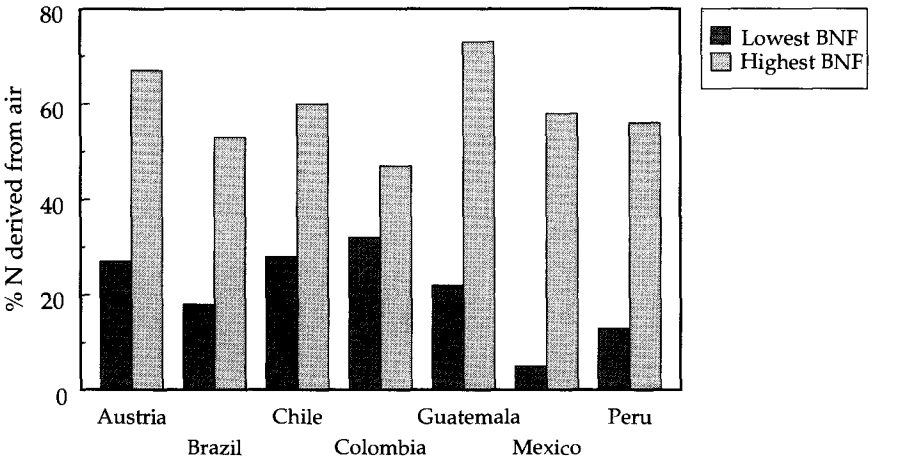


Source: Data of Pena-Cabriales, Mexico, See: Hardarson et al. 1993.

Figure 5. Variation between common bean cultivars in nitrogen fixation under field conditions in Mexico.

devoid of effective indigenous rhizobia. Countries with less developed inoculant industry may have to establish or improve their production. Quality assurance will be essential as it is very difficult for farmers to evaluate the effectiveness of inoculants.

Compared to the number of microbiological studies to enhance BNF in grain legumes, very little attention has been given by plant breeders to this trait. It is well known that grain legume species vary greatly in terms of their capacity for N_2 fixation (Fig. 4). Some are very efficient (e.g. faba bean), others are intermediate (chickpea, cowpea and soybean) and some are inefficient (e.g. common bean). The main objective of the CRP on common bean was to study the variation of this species in N_2 fixation supportive traits (Figs. 5 and 6). When investigated under field conditions, large variation in N_2 fixation was found between common bean cultivars (Bliss and Hardarson, 1993). Although fixation was generally low, some cultivars were efficient at supporting the N_2 fixation process. These cultivars could then be recommended directly to farmers or to plant breeders for the transfer of these traits into other cultivars.

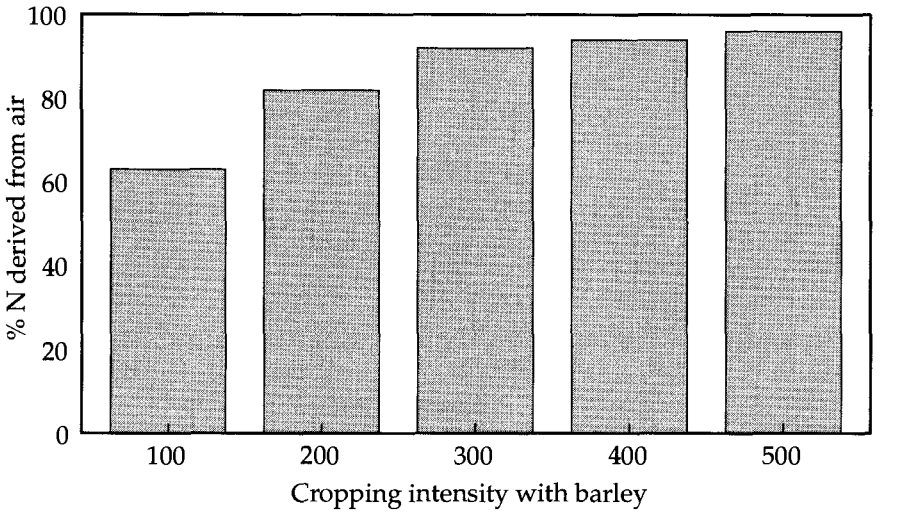


Source: Hardarson et al. 1993

Figure 6. Range in nitrogen fixation in common bean under field conditions. (Approximately 20 cultivars were tested in each country).

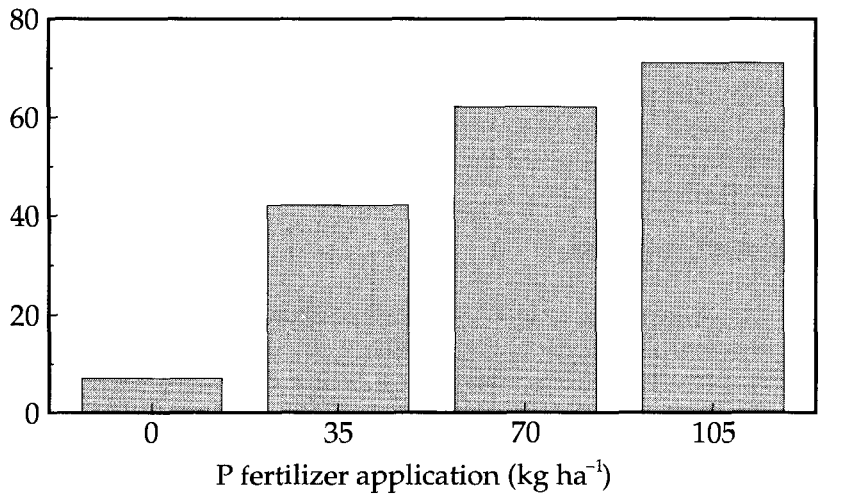
Agronomic methods may also be used to enhance BNF in grain legumes. Cropping intensity with cereals has been reported to affect N_2 fixation (Fig. 7). Legumes grown in mixed cropping with cereals usually fix a higher proportion of their nitrogen than legumes cultivated as mono-crop. This, however, may not be reflected in higher yields. Similarly, phosphorus or water availability may affect N_2 fixation (Figs. 8 and 9). All cultural conditions

have to be optimized for maximizing N_2 fixation in grain legumes under field conditions.



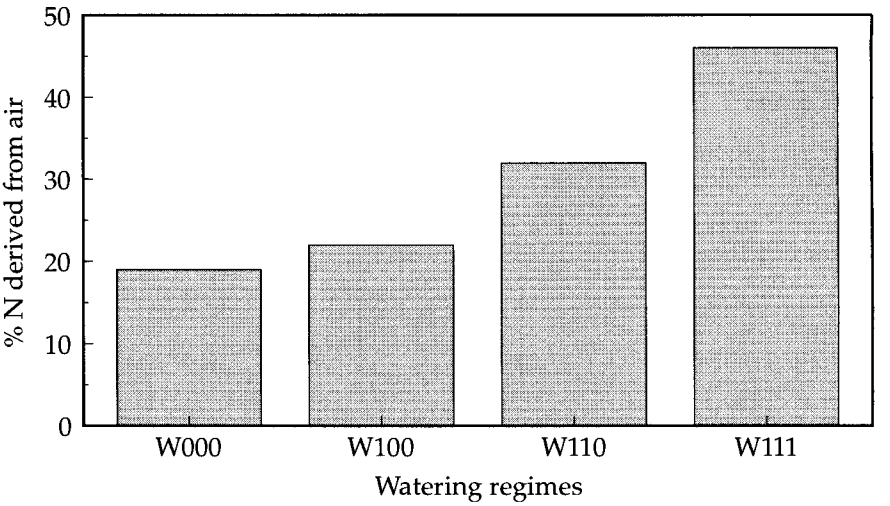
Source: Danso et al. 1987.

Figure 7. The proportion of N derived from atmosphere in faba bean as affected by mixed cropping with barley under field conditions. Pure faba bean (100) and increasing competition from barley rows (200-500).



Source: Data of J. Dombovari, Hungary. See Zapata and Baert 1989.

Figure 8. Nitrogen fixation in soybean as affected by phosphorus application under limiting soil P conditions.



Source: Kirda et al. 1989.

Figure 9. The effect of water stress (0) during maturity (110), reproductive and maturity stages (100), and whole growing season (000) on nitrogen fixation in soybean. The control received no stress (111).

Preliminary data obtained at the FAO/IAEA laboratory using ^{15}N stem labelling techniques indicate the importance of below ground N (BGN) of legumes for succeeding crops. Recent data of Khan et al. (2002) based on the measurements of ^{15}N of harvested plant parts and root-zone soil suggested that BGN represents 39% of total plant N for faba bean, 53% for chickpea, 20% for mungbean and 47% for pigeonpea. It is clear also from the results obtained at the FAO/IAEA laboratory that BGN has been largely underestimated in the past, as also the N contribution of legumes in various cropping systems.

RECOMMENDATIONS

There are many ways of enhancing N_2 fixation in grain legumes. The experience obtained in the FAO/IAEA program indicated that the largest gains could be obtained by selecting/breeding grain legumes for N_2 fixation supportive traits. Concerted efforts are required in this area of research by CG centres and other international organizations. Inoculant production and quality assurance is essential in any project on BNF and a major effort should be made to ensure availability of inoculants in many developing countries. To be able to enhance BNF in grain legumes a method for the quantification of BNF in various cropping systems is essential. The FAO/IAEA has extensive experience in measuring BNF under field conditions using ^{15}N tracer

technology, and the assistance of the FAO/IAEA may be particularly useful for the implementation of the Challenge Programme on BNF being planned by the CG centers.

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9

Vegetable Legumes – A Source of Increased Productivity, Improved Soil Fertility and Nutritional Health[1]

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ABSTRACT

The Asian Vegetable Research and Development Center (AVRDC) deals mainly with two legume vegetables: mungbean and vegetable soybean. While mungbean production is concentrated in hot and dry areas of Asia, vegetable soybean is spread from Asia to Africa and USA. Both crops have a short growing period, and excellent potential to contribute to nitrogen fixation, sustained soil fertility and nutritional health. AVRDC mungbean lines are very early maturing (55-65 days) and high yielding (2.5 t ha^{-1}), rich in bioavailable iron (up to 12 mg/100g) and rich in protein. In addition, the unit price of protein and iron in mungbean is very low compared to that of other commodities. Due to its short maturing time, mungbean can be easily integrated into cereal cropping systems. Since 1972, mungbean production growth rates in Asia have been more than 10% annually. Vegetable soybean has similar potential – early maturing (75-85 days), high yields (7 t ha^{-1} fresh vegetable), and year-round growing potential (as AVRDC lines are photo insensitive). Soybean provides plant protein of high quality, high quality oil, and other health-improving chemicals (isoflavones, tocopherol). Future research requirements are (1) better understanding of soil fertility effects by mungbean and vegetable soybean, and sustainability of soil fertility and long-term impact to follow-up crops; (2) optimizing integration of mungbean and vegetable soybean into cereal systems under different environments; (3) understanding limiting socioeconomic factors, water and labor requirements; (4) increasing yield and nutritional quality traits of mungbean and vegetable soybean; and (5) assessment of farmer's and consumer's gains, and analysis of changes in consumption pattern, including gender-specific nutritional impact.

[1] AVRDC Position Paper

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INTRODUCTION

The Asian Vegetable Research and Development Center (AVRDC) deals mainly with two legume vegetables, mungbean and vegetable soybean. While mungbean production is concentrated in Asia in hot and dry seasons, vegetable soybeans are spread from Asia to Africa and the USA. Both crops have a short growing period, combined with excellent yield potential to contribute to N_2 fixation and sustained soil fertility and to improve human nutrition.

POTENTIAL OF MUNGBEAN

Early Maturity and High Yield

AVRDC lines mature in only 55-65 days, and are resistant to mungbean yellow mosaic virus (MYMV), a major disease of mungbean. They also now synchronously mature, and give high yields of up to 2.5 t grain ha^{-1}, while local lines do not yield beyond 0.5-0.8 t ha^{-1}.

Rich Source of Iron and Protein

Mungbean is very rich in iron, and boiling the bean with amino acid rich vegetables like tomato doubles the bioavailability of iron to 12 mg/100 g (Table 1). This is comparable to meat with iron contents of 13-15 mg/100 g.

Table 1. Improved bioavailability of iron from mungbean.

	Bioavailability of iron (mg/100 g)
Traditional recipe	4.0-5.8
Improved recipe (with tomato or carrot)	10.1 and 11.3

Source: Amirthaveni Subramanian and Yang 1998

Thus, mungbean could substantially contribute to alleviation of iron deficiency, especially in countries like India where most of the population is vegetarian, and would benefit from a rich iron source. People all over the world suffer from iron-deficiency anemia, the effects of which are often severe (Table 2).

Table 2. Global incidence of iron deficiency.

• 3.5 billion people are affected (about 40-50% of the world population.)
•The most seriously affected are: —Children with an age between 6 months to 2 years —Pregnant women —Lactating mothers —Population below the poverty line —88% of the population in Inda

Source: ACC/SCN/ IFPRI 2000

Increased Productivity and Soil Fertility

The integration of mungbean into cereal farming systems has the following advantages:

- Due to the short maturing period of only about 60 days, an extra mungbean crop can be grown each year in rotation with cereals, especially after wheat harvest in the rice-wheat cropping systems of the Indo-Gangetic plains that cover more than 10 million ha, using the residual soil moisture in fallow land following a cereal crop.
- In addition, the follow-up wheat crop shows a yield increase of about 20%. The calculated additional gain in Pakistan accumulated to US$25 million annually and about half of this can be attributed to improved soil fertility (Ali et al. 1997).

More information is required on the effect of soil fertility parameters and N_2 fixation resulting from the integration of mungbean into the cropping system. The effect on yield increase of the follow-up wheat crop, however, provides a clear indication of improved soil fertility.

MUNGBEAN PRODUCTION TRENDS

Average annual growth rates for mungbean and pulses from 1972-2000 are shown in Table 3.

Table 3. Annual growth rates of mungbean in Asia, 1997-2000.

	Mungbean % growth	Total pulses % growth
Area	+ 13.3	+ 4.7
Yield	+ 4.2	+ 1.7
Production	+ 17.3	+ 6.4

Source: Weinberger 2002.

Myanmar and China showed very significant increases in mungbean production (Table 4). As a result of the increase in area and yield, total production in Myanmar has grown 54-fold between 1972 and 1997, and Myanmar has become the main exporter of mungbean. Based on the data available, it is seen that mungbean production in China was also increased by 71% within a period of only three years (1997 to 2000).

Table 4. Increase in mungbean production in Myanmar and China.

	Myanmar			China		
	1972	1997/2000	% increase	1997	2000	% increase
Area (ha)	34,020	650,581	+2,000	531,381	772,098	+45
Yield (kg ha^{-1})	244	826	+238	979	1,154	+18

The mungbean green revolution in Pakistan has also been a great success and helped put farmers, consumers, and the environment all on the winning side (Ali et al. 1997).

Figure 1 shows the share of mungbean production in increase in overall pulses production in several countries.

Costs of Protein and Iron from Selected Food

Mungbean and other pulses are cheap sources of protein and iron; therefore, they are easily affordable by the poor (Table 5).

ADVANTAGES FOR FARMERS

The advantages that farmers would gain by mungbean production include:

- Production of an additional crop under unfavorable environmental conditions (lack of water, hot/dry climate)
- Breaking the cereal-cereal cropping system for better soil fertility
- Diversifying the cropping system and farm income with a legume (with high demand elasticity beyond 0.50)
- Improved nutrition (iron/protein)

Current Research

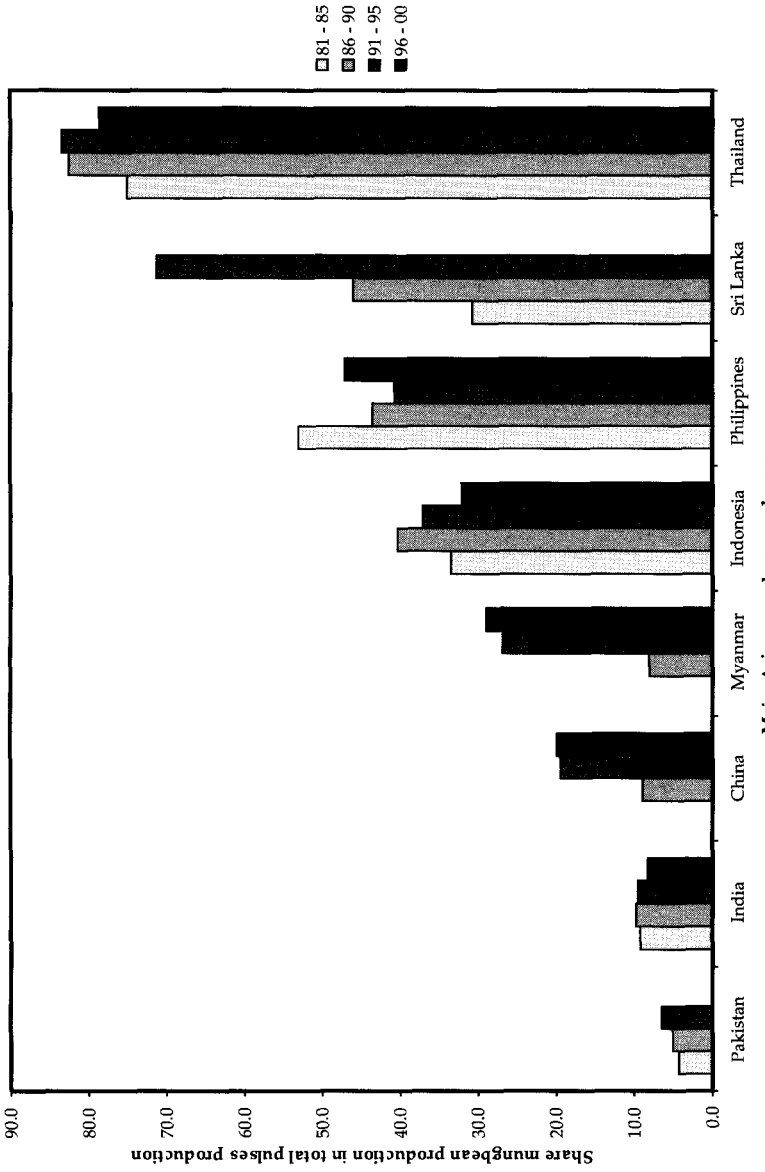
The current research is focused on integration of mungbean into the Indo-Gangetic rice/wheat cereal system jointly with the International Maize and Wheat Improvement Center (CIMMYT) and the International Rice Research Institute (IRRI). The tasks are:

- Cultivar identification
- Technology development for effective resource management
- Evaluation of the effect of mungbean (soil productivity)
- Determination of water use efficiency

Table 5. Average price (in Indian Rs) for one unit of protein and iron from selected food items.

Food item	Mean price/ kg	Mean price per g protein	Mean price per mg iron
Meat *(average quality)*	104.0	0.56	11.72
Fresh fish *(average quality)*	42.1	0.28	14.03
Mungbean (*Vigna radiata*)	25.5	0.11	0.53
Red gram (*Cajanus cajan*)	26.0	0.34	1.73
Black gram (*Phaseolus mungo*)	24.9	0.10	0.66

Source: Weinberger 2002



Source: Weinberger 2002.

Figure 1. Share of mungbean production in overall pulses production in the major Asian mungbean-producing countries.

- Seed production and distribution
- Recipe development
- Impact assessment

POTENTIAL OF VEGETABLE SOYBEAN

Early Maturity, High Yield and Photo Insensitivity

AVRDC vegetable soybean lines mature in only about 75-85 days, are resistant to major diseases, and photo insensitive, so they can be grown round the year. They yield up to 7 t ha^{-1} fresh vegetable pod (graded).

As can be seen in Table 6, vegetable soybeans are rich in high quality plant protein and contain high quality oil (cholesterol free) and other health-improving chemicals (folic acid, isoflavones, tocopherol).

Easy Preparation for Consumption

Vegetable soybeans are easy to cook – the pods should be boiled for 5-7 minutes in an open container, and the green beans eaten. If the pods are kept too long in the water,they turn brown and do not look very appetizing. If removed in exactly 7 minutes, they retain the bright green color (Shanmugasundaram, 2002).

Increased Productivity and Soil Fertility

The integration of soybean into cereal farming systems has the following advantages:

- Easier integration, because of short maturing period (75-85 days)
- N_2-fixation about 120 kg ha^{-1}, in addition to green leafy residues for integration into soil (Shanmugasundaram, 2002)

Advantages to Farmers

It is advantageous to farmers to grow vegetable soybean because:

- Vegetable soybean is a nutritious vegetable which can supplement cereal based diet of rural people
- Vegetable soybeans can bring additional cash income to farmers
- Since vegetable soybean is a legume, it can break the cereal-cereal cropping system
- Vegetable soybeans can produce a total biomass of up to 40 t ha^{-1}, of which 25% is green pods. The remaining 75%, consisting of leaves,

Table 6. Composition of edible protein, minerals and vitamins of vegetable soybeans and green pea.

	Vegetable soybean	Green pea
Energy (kcal)	139.0	94.0
Moisture (%)	68.2	75.6
Protein (%)	13.0	6.2
Fat (%)	5.7	0.4
Total carbohydrate (%)	11.4	16.9
Crude fiber (%)	1.9	2.4
Ash (%)	1.7	0.9
P (mg/100 g)	158.0	102.0
Ca (mg/100 g)	78.0	32.0
Fe (mg/100 g)	3.8	1.2
Vit. A (ß carotene eq.) (mg/100 g)	360.0	405.0
Vit. B_1 (mg/100 g)	0.4	0.3
Vit. B_2 (mg/100 g)	0.2	0.1
Vit. C (mg/100 g)	27.0	27.0

Source: FAO 1972.

stem and roots, are returned to the soil. The dry matter yield of the residue of vegetable soybeans can be 6.0 to 6.6 t ha^{-1} in 80 to 87 days compared to 5.0 t ha^{-1} for *Crotalaria* and *Sesbania*. The total amount of N, P and K in the residue is about 170 kg N, 18 kg P and 150 kg K ha^{-1} (AVRDC 1998). It is better than that of green manure crops. Since the growth cycle of vegetable soybeans from sowing to harvest is about 75-85 days it is similar to a green manure crop. In addition, the shells from the pods can be fed to cattle.

- Vegetable soybeans have large seeds. Large-seeded soybeans usually fetch a premium price in the world market. Furthermore, vegetable soybean seeds are more expensive compared to grain soybeans. In Asia, for example, grain soybeans cost around US$ 0.25 kg^{-1}. However, vegetable soybean seed costs around US$ 3 to 4 kg^{-1}.

Production Trends

- Production initially started in Taiwan with AVRDC bred line KS1 to serve the Japanese market. The proportion of that line rose from 46.6% in 1988 to 91% in 1994. Meanwhile, production, because of high production costs, moved to Thailand and China.
- While in the 1980s only a few countries produced vegetable soybean, there are now 31 varieties released in 11 countries, including USA. A set of 120 AVRDC lines has been given to 57 countries for evaluation (Fig. 2); however, detailed production figures are not known. As shown in Figure 2, AVRDC vegetable soybeans are widely evaluated

Source: Shanmugasundaram 2002.

Figure 2. AVRDC Vegetable Soybeans: Evaluation, commercial production and export in the world as of 2001.

The above illustration is a rough sketch showing soyabean production in the world and it does not purport to depict political boundaries. Thus the boundaries shown may not be correct or accurate.

around the globe and rapidly being extended to farmers to improve their income and nutrition.

Current Research

Current research thrusts of vegetable soybean at AVRDC include developing narrow leaflet type (high percent of 2 and 3 seed pods), lipoxygenase null (less beany flavor), glabrous types (resistant to pod borers), better taste (higher percentage of sucrose), special flavor (unique taro flavor type), seeds of various colors (diversity for consumers) and richness of functional nutrients (high isoflavones, tocopherol or folic acid) (Shanmugasundaram and Yan 2001).

REQUIRED FUTURE RESEARCH

Future research areas that need addressing are:

(1) Nitrogen fixation by mungbean, especially the N_2 fixation rate in different environments, the effect of *Rhizobium* inoculation of mungbean seed, and low efficiency of N_2 fixation can be increased.
(2) The effects of soil fertility of growing legumes, especially how the main soil fertility parameters are affected, the sustainability of the effect, and its long-term impact on following crops.
(3) Integration on mungbean and vegetable soybean into cropping systems, with special reference to the optimal integration into the cropping sequence and the best cultural practices under different environments. How this affects water and labor requirements and farm income, and the socioeconomic limitations to arrive at efficient integration.
(4) Yield and quality improvement, which could include raising on-farm yield above 1 t ha^{-1} (from the present 300 kg ha^{-1}) with mungbean yellow mosaic virus (MYMV)-resistant lines. Also, raising protein quality by transfer of specific traits from black gram (Table 7), selection of mungbean lines rich in iron, and transfer of bruchid-resistant traits from black gram to mungbean.

Table 7. Methionine content in mungbean and black gram.

Entry	Methionine content (mg/100g)
Mungbean	
VC 1973A	8.1 ± 1.0
V 2709	27.0 ± 2.2
Black gram	
VM 3171	100.7 ± 9.0
VM 2164	114.9 ± 2.4

Source: Shanmugasundaram 2002

(5) Yield and quality improvement for vegetable soybean would involve raising vegetable soybean on-farm yield to 7-8 t ha^{-1} (graded pods), and selection of vegetable soybean with high isoflavones and tocopherol contents (+100%).

(6) Impact assessment. Assessments are required for changes in farm income and consumer's gains, including iron uptake, through new technologies in main producer countries, changes in consumption pattern of different income groups, and development impacts, including nutritional and gender-specific improvements in main producer countries.

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II

Future Prospects of Advanced Research on SNF in ARIs

10

The Importance of Biological Nitrogen Fixation by Trees in Agroforestry

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ABSTRACT

The introduction of trees that symbiotically fix atmospheric nitrogen is widely acknowledged as one of the most efficient means to sustain the productivity of agrosystems through the improvement of the soil nitrogen balance, especially in the tropics. Nevertheless, very few research programs specifically focus on this topic. Quite varied results have been obtained on fixed-nitrogen enrichment of the soil and use of this nitrogen to fertilize annual intercrops. The N_2-fixing potential of tree species is often low or inhibited by environmental constraints such as drought, salinity, and excess mineral nitrogen or plant diseases. The symbiotic bacteria/host tree association could possibly be improved through plant biotechnology and genetic engineering research. Symbiotic nitrogen fixation could also be optimized by modifying current agricultural practices. Nitrogen-fixing trees can be intercropped or not with other annual crops in various conditions, e.g. hedges, bush fallows and long-term rotations. Such management programs should be better integrated in rural environments through the promotion of multipurpose species producing wood and high-protein livestock fodder as well as edible fruits or seeds.

INTRODUCTION

A large majority of nitrogen (N_2) fixing trees are members of the *Leguminosae* family and are symbiotically associated with rhizobia, while a small number of species are actinorhizal plants belonging to different plant families and

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associated with *Frankia*, another type of soil bacteria. It is not clear whether this capacity of trees to fix the atmospheric nitrogen is only utilized for their own N_2 requirements or also at least partially for that of the associated crops. Field experiments and *in situ* observations showed contradictory results for either a beneficial effect of tree legumes on the growth of associated crops or soil impoverishment (Lundgren 1978). These opposite results are due to various factors. All leguminous species do not fix nitrogen (de Faria et al. 1989, Sprent 2001), such as the majority of trees belonging to the *Caesalpiniaceae* sub-family with only 23% of nodulating species described (*vs* 90% in the *Mimosoideae* and 97% in the *Papilionoideae*). On the other hand, nodulated species sometimes exhibit very high genotypic variations in their N_2-fixing potential. Nitrogen fixation is particularly sensitive to the environmental constraints that can limit or even inhibit this process. The nitrogen balance of a given ecosystem including N_2-fixing species can be negative when the quantity of N exported through harvesting is higher than N inputs. Our summarized review presented below is focused on N_2 fixation and N transfers by trees in agroforestry and forest ecosystems.

BIOLOGICAL NITROGEN FIXATION BY TREES

It is essential to determine as precisely as possible the N input in any ecosystem including a N_2-fixing species. Different methods are available to measure this input – balance, methods by difference, isotopic methods (isotopic dilution, natural abundance in ^{15}N, A-value method), nodulation observation, ureides and amides in xylem sap and acetylene reduction method (Peoples et al. 1989, Danso et al. 1992). Although none of these methods is perfect, the combination of several of them should enable the process to be quantified with a sufficient degree of precision. The most accurate methods to quantify biological nitrogen fixation (BNF) are the isotopic ones. However, many sources of variation as well as the own characteristics of trees induce quite high inaccuracies in the evaluation of BNF (Danso et al. 1993, Boddey et al. 2000). For instance, in all the isotopic methods used, the proportion of ^{15}N contained in a non-fixing reference plant is used to calculate BNF. Since it is supposed as a prerequisite that both N_2-fixing and non-fixing tree species explore the same soil horizons – due to similar types of root system architecture – and assimilate the same sources of N, the choice of the reference plant is crucial for evaluating BNF, which can vary considerably according to the selected reference tree.

The quantity of N_2 fixed varies dramatically according to the soil and climatic conditions. The N_2-fixing potential (NFP) is defined as the maximal N_2-fixing activity of a given species that is expressed in the absence of any limiting factor. When a limiting factor occurs, the N_2 fixation is reduced and the actual N_2 fixation (ANF) is measured.

Potential Fixation

The NFP depends on the genetic characteristics of the associated bacterial strain and those of the host plant. Based on the results published on the nodulation and quantity of N_2 fixed by the most important planted species, Ganry and Dommergues (1995) ranked them according to their NFP. Among species with a high NFP, i.e., those that were estimated to fix between 60 and 100 kg N $ha^{-1}yr^{-1}$, are: *Leucaena leucocephala, Calliandra spp., Acacia mangium, Acacia auriculiformis, Acacia crassicarpa, Acacia mearnsii, Gliricidia sepium, Sesbania* spp., *Casuarina equisetifolia* and *Casuarina cunninghamiana.* On the other hand, species such as *Prosopis juliflora* and *Acacia saligna* (syn.: *A. cyanophylla*) are considered as having an intermediate NFP while *Acacia raddiana, Acacia senegal, Acacia cyclops* and *Faidherbia albida* have a low NFP.

Actual fixation

The ANF measured in trees has often been overestimated. The quantities of N fixed are in general lower than 30-50 kg ha^{-1} yr^{-1} (Sutherland and Sprent 1993). Different environmental factors, such as phosphorus deficiency, drought, acidity or alkalinity of soil and excess of soil N (enhancing tree growth but inhibiting N_2-fixing activity), affect the N_2-fixing activity of trees *in situ*. Some observations suggest that, in a closed ecosystem, N_2 fixation tends to decrease with plantation ageing, as a consequence of the progressive accumulation of available N. This accumulation could generate the decline in nodulation that is often observed in old plantations. In addition, it is likely that the duration of fixation activity in plantations varies greatly according to the tree species, tree density and climatic and soil conditions. However, N_2-fixing activity was observed in old plantations such as in *Casuarina equisetifolia* in Senegal (Ganry and Dommergues 1995).

Actually, the quantification of fixed nitrogen using isotopic methods has been performed on very few leguminous tree species under field conditions, as shown in Table 1, which summarizes most of the studies available in the literature on this subject. This lack of information can be attributed to the particularly difficult sampling that has to be implemented in trees considering their high biomass and size. Accordingly, most of the studies reported in Table 1 were performed on one or two year old trees whereas BNF (%Ndfa) by older trees was only estimated from leaf sampling.

Specificity of Nodulation in Trees

In contrast to annual and herbaceous legume species, the nodulation in trees can be perennial and deep. Many actinorhizal species such as *Casuarina equisetifolia*, as well as woody legumes, bear perennial nodules. This is an

Table 1. Estimation of nitrogen fixation by different tree species in plantation conditions using isotopic methods.

Species	Location	Plantation age[a] (years)	%Ndfa[b]	Total N fixed (kg ha^{-1})[a]	Isotopic method used[c]	References
Acacia caven	Chile	1	14	0.5	ID	Ovalle et al. 1996
		2	86	9		
Acacia holosericea	Senegal	10	39	nd	NA	Ndiaye and Ganry 1997
Acacia mangium	Côte d'Ivoire	2	50	nd	NA	Galiana et al. 2002
Acacia melanoxylon	Australia	2.25	43	< 1	NA	Hamilton et al. 1993
Acacia mucronata	"	"	48	"	NA	"
Alnus glutinosa	France	> 15	94	nd	NA	Beaupied et al. 1990
Alnus incana spp. *rugosa*	U.S.A.	nd	85-100	43 yr^{-1}	NA	Hurd et al. 2001
Alnus incana	France	5-6	75	nd	NA	Domenach et al. 1989
Calliandra calothyrsus	Australia	1	50	76	NA	Purwantari et al. 1996
Casuarina equisetifolia	Puerto Rico	2	42-67	82-94 yr^{-1}	ID	Parrotta et al. 1994a
"	Senegal	3	38	15 yr^{-1}	NA	Mariotti et al. 1992
Erythrina lanceolata	Costa Rica	1	0-53	0-72	NA	Salas et al. 2001
Faidherbia albida	Senegal	1	15-23	nd	ID	Gueye and Ndoye 2000
Flemingia macrophylla	Burundi	1	-	10	NA	Snoeck 1995
Gliricidia sepium	Senegal	10	0-17	nd	NA	Ndiaye and Ganry 1997
Hardwickia binata	"	"	0-22	nd	NA	"
L. leucocephala	Puerto Rico	2	70	103 yr^{-1}	ID	Parrotta et al. 1994b
"	Nigeria	3	62-75	98-119 yr^{-1}	ID	Sanginga et al. 1996
Prosopis alba	Chile	1	25	0.4	ID	Ovalle et al. 1996
		2	52	1.8		
Prosopis chilensis	"	1	31	0.5	ID	"
		2	70	2		
Prosopis cineraria	Senegal	10	21	nd	NA	Ndiaye and Ganry 1997
Prosopis glandulosa	USA	1	41-63	40	NA	Shearer and Kohl 1991
Robinia pseudoacacia	Austria	2	90	110 yr^{-1}	ID	Danso et al. 1995

[a] nd : not determined

[b] %Ndfa : Percentage of nitrogen derived from the atmosphere

[c] ID : Isotopic dilution with application of ^{15}N fertilizer ; NA : Natural abundance in ^{15}N

advantage since the N_2-fixing activity can start again rapidly without formation of new nodules when suitable conditions are met. A deep nodulation was observed in some phreatophytic tree legume species such as *Prosopis juliflora* (Felker and Clark 1982), that allows them to keep fixing N_2 even when the higher horizons of soil are dry.

NITROGEN TRANSFERS FROM N_2-FIXING TREES TO SOIL AND ASSOCIATED CROPS

Very few studies about the quantification of N transferred from N_2-fixing species to non-fixing species have been done so far, especially in trees. The measurement of indirect transfer – i.e. through litter recycling – is very difficult to implement in field conditions since marked N used for this purpose has strictly to be applied to the N_2-fixing species. In the same way, the measurement of direct transfer – i.e. from root to root involving or not mycorrhiza – has never been demonstrated in situ (Dommergues et al. 1999). However, although it was not quantified, the transfer of N_2 fixed from a N_2-fixing tree to non-fixing plants was clearly showed by Van Kessel et al. (1995) using the ^{15}N natural abundance method. The $\delta^{15}N$ of *Leucaena leucocephala* and that of understorey non-fixing plants decreased progressively and concomitantly from the first to the sixth year of plantation due to the recycling of the litter deposited by the legume tree.

Modes of Transfer

Without the use of tracing isotopic methods, the transfer of N_2 fixed in the different compartments of the ecosystem is difficult to follow up due to the different processes interfering together. Practically, these transfers are evaluated according to the fluctuations in the soil N content or those observed in the production yield of associated annual crops.

Partial N Recycling

All tree species are able to recycle the mineralized N issuing from the organic litter (leaves, twigs, branches, flowers and fallen fruits) and root residues (root exudates, nodules) that are decomposed in soil by macro- and microfauna and microorganisms. Leaching along the trunks, stems and leaves (stem-flow) has to be integrated to the N-flow. Some studies suggested that the proportion of recycled N in N_2-fixing trees could be lower than that found in non-fixing ones (Wheeler 1991).

Internal Transfers

Internal transfers of N from N pools can be important during vegetative regrowth, flowering or fruiting.

Redistribution of N in the Soil Profile

Nutrients and mineral N are taken up by trees from deep horizons and brought to the soil surface. Phreatophytic species sometimes take up nutrients from 30 m depth (Dupuy and Dreyfus 1992). Among the different elements assimilated by the roots, phosphorus is one of the most important, especially when trees are mycorrhizal. The symbiotic fungi colonizing the root system (endo- and ectomycorrhiza) help the plant to mobilize different elements, P in particular (Wheeler 1991).

Examples of N Transfer from the Trees to the Soil

The studies focused on the impact of N_2-fixing trees on soil enrichment in N give very variable results that largely depend on the experimental and natural conditions.

Unexploited Forest Plantations

In a sodic soil in India, Garg and Jain (1992) found that 8 years after planting, the soil N content was multiplied by 4 under an *Acacia nilotica* stand and by 6 under *Prosopis juliflora*. In Senegal, the soil N pool under a 13-year-old *Casuarina equisetifolia* stand reached 309 kg ha^{-1} whereas that of the same original soil in close vicinity but without tree establishment was only 80 kg ha^{-1} (Dommergues 1963).

Industrial Forest Plantations

In such forest plantations for biomass production, the N balance at the end of culture rotation is variable according to the situations. In South Africa, Orchard and Darby (1956) found that the soil N content of 6 different sites planted with *Acacia mearnsii* rose from 0.35 to 0.53% after 30 years of culture (i.e. after three rotations) when compared to 6 unplanted control sites, which is equivalent to 180 ± 40 kg ha^{-1} yr^{-1}. On the other hand, the N balance of soils planted with *Acacia mangium* can be negative when log harvesting induces more exportation of N than N input through biological fixation. In Malaysia, Halenda (1989) showed that the total aerial biomass of a seven year-old *A. mangium* stand contained 616 kg N ha^{-1}. The quantity of N exported through log harvesting corresponded to 284 kg ha^{-1} after 6 years of planting. But other processes of N loss have to be taken into consideration in the net balance,

such as leaching, erosion and drainage, volatilization of ammonium-N and denitrification, that could amount to 10-20 kg ha^{-1} yr^{-1} (Wetselaar and Ganry 1982, Ganry 1990). In other terms, a minimal N input of 50-60 kg ha^{-1} yr^{-1} due to N_2 fixation is needed to obtain an equilibrated N balance in the absence of N fertilization and when exportation is restricted to log harvesting.

Trees Associated with Annual or Perennial Crops

The N_2-fixing trees can contribute to the N supply of associated annual crops. In the particular case of observations made after forest clearing, this input is not sufficient to prevent the soil N content from decreasing. This was observed in Nigeria (Lal 1989), where the soil N content was 0.214% in the 0-5 cm horizon and 0.134% in the 5-10 cm horizon immediately after exploitation of a 5-year-old secondary forest. After 4 years of culture with maize and cowpea (*Vigna unguiculata*) and without adjacent hedgerows of *L. leucocephala*, the soil N content dropped to 0.038 and 0.042% in the 0-5 and 5-10 cm horizons respectively. By contrast, the introduction of *L. leucocephala* hedgerows with application of prunings on the cultivated soil limited the reduction of soil N content in the 0-5 and 5-10 cm horizons since they reached 0.103 and 0.090% respectively. N losses were probably due to the exportations of the successive cultures of maize and cowpea as well as to physical or microbiological processes (such as leaching and denitrification).

In another situation, Sanchez (1987) supposed that the relatively high level of fertility found in the close vicinity or under the canopy of trees that grew spontaneously was anterior to their establishment. The same hypothesis was proposed by Geiger et al. (1992) for *Faidherbia albida* in Sahelian regions, this tree species being known as beneficial to the yield of annual crops such as millet (*Pennisetum glaucum*), peanut (*Arachis hypogea*) or sorghum (*Sorghum bicolor*). However, accurate field experiments are needed to confirm these assumptions.

Examples of N Transfer from Trees to Associated Crops

The N transfer from trees to crops was evaluated (von Carlowitz 1989, Giller 2001) in the following agroforestry systems.

Alley Cropping Systems

In these systems, the crops are established between hedgerows of trees, mostly leguminous species such as those of the genera *Erythrina*, *Gliricidia*, *Inga*, *Leucaena*, *Mimosa*, *Robinia* and *Sesbania*. In such systems, the trees are pruned at regular time intervals and the prunings obtained are deposited as mulch or incorporated as green manure. In Australia, Xu et al. (1993) showed that this practice improved significantly the yield of maize cultivated between

hedgerows of *L. leucocephala* separated by 4.5 m. In Nigeria, at the station of the International Institute of Tropical Agriculture (IITA, Ibadan), Sanginga et al. (1988) observed that a part of the N_2 fixed by *L. leucocephala* in the same system of culture is transferred to maize through pruning application. The gain was equivalent to the addition of 80 kg ha^{-1} of N fertilizer when *L. leucocephala* was inoculated with an efficient *Rhizobium* strain or equivalent to the addition of 40 kg N ha^{-1} without inoculation. The uptake of N issued from prunings by the associated crops is often low. As shown by Mulongoy (1983) in a study carried out in southern Nigeria, prunings from *L. leucocephala* applied to maize produced 300 kg N ha^{-1} that corresponded to the application of 30 kg ha^{-1} of ammonium sulfate alone. Such a low efficiency could be explained by the fact that the leaves and branches rapidly released their N (50% of the total amount during the first month after application) after mineralization and losses due to volatilization or leaching. Another part of the N_2 fixed is transferred to the associated crops through leaf and root (including nodules) litters. In the latter experiment mentioned, Mulongoy (1983) estimated that the proportion of N transferred through this way would be approximately the same as the one issued from prunings.

Faidherbia albida or Prosopis cineraria Parks

Faidherbia albida (Sahelian regions) and *P. cineraria* (India) parks are considered very efficient agroforestry systems. The productivity of crops established under the trees is significantly higher than that recorded outside (CTFT 1988). However, in the Sahelian zone, the PNF or ANF of *F. albida* is known to be lower than that of several *Acacia* species (Schulze et al. 1991) in accordance with the usual low nodulation observed in these dry climatic conditions. The higher fertility level observed under *F. albida* is probably not due to the symbiotic fixation but rather to other factors such as the input of organic matter deposited by the cattle grazing under the tree shade.

Improved Forest Fallows

An improved forest fallow consists of a plantation of N_2-fixing trees inserted in the culture cycle. Such an agroforestry system is the most realistic and promising for application on a large scale. Anderson and Sinclair (1993) described a system experimented with in the Sahelian zone: *Acacia senegal* was first cultivated for ten years. After burning the tree plantation, sorghum was cultivated for 1 or 2 years. The soil was left under fallow afterwards and the spontaneous regeneration of *A. senegal* occurred for a new cycle. An experiment carried out by the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD, France) in Côte d'Ivoire (West Africa) aimed at comparing the effect of four fallows of legume trees (*Acacia mangium*, *Acacia auriculiformis*, *Albizzia lebbeck* and *L. leucocephala*)

and a natural herbaceous fallow (*Chromolaena odorata*) on the total soil N content and maize yield (Oliver and Ganry 1994). Four years after being planted, the forest fallow had no major effect on either. However, the *A. lebbeck* fallow had a significant and positive effect on the fertilizer efficiency when the mulch from tree leaves was not burned.

IMPROVEMENT OF BIOLOGICAL NITROGEN FIXATION

The field trials carried out in many tropical countries show that, except in a few cases, the ANF is not sufficient to ensure the maintenance of the N soil pool when crops or forest products are harvested. Different strategies were proposed to improve the BNF (Dreyfus et al. 1988, Danso et al. 1992, Hardarson 1993).

Improvement of the Host Tree

The improvement of the host plant can occur at different levels – the ability to fix N_2 at a given soil N threshold; the root system architecture of the tree; and under conditions of prior inoculation.

- The threshold of available soil N inhibiting N_2 fixation varies according to genetic differences in the host plant (Herridge and Betts 1988). Although very little data is available on this aspect in trees, some species, such as alder (Domenach et al. 1989, Wheeler 1991), seem to keep fixing N_2 in these conditions. New studies on this aspect would allow identification of the tree species, provenances, or clones 'tolerant' to the available soil N.
- The architecture of the root system is an essential factor to be considered in the management of agroforestry systems since it determines the nutritional competition between the trees and associated crops. Since its root system is characterized by a tap-root, *F. albida* does not compete with adjacent cultures of peanut, millet or cowpea, whereas trees with a superficial root system, such as *Acacia nilotica* and *Acacia tortilis*, have a negative effect on productivity of the adjacent crops. A decrease of 50% in productivity can be obtained up to a distance of 2.5 m from the tree hedge. With *P. juliflora*, the competition is minimized and does not cause a decline in productivity of the associated crops (Cazet 1989).
- Some authors suggest the selection of highly promiscuous host plant genotypes, i.e. spontaneously nodulating with many indigenous rhizobium strains, which would not require any bacterial inoculation. Another more applied strategy is to select host plant genotypes specific to a given effective strain (Hardarson 1993).

More generally, the improvement of the host tree can be obtained following a traditional selection approach through the screening of suitable genotypes after breeding, selection of provenances or vegetative propagation of elite trees (Sougoufara et al. 1992).

Improvement of the Bacteria and Inoculation Techniques

The improvement of the bacteria to be inoculated is done by screening and selecting the most efficient strains among a collection of wild strains isolated from the host plant. The infectivity and effectiveness of these strains have firstly to be tested in controlled conditions in vitro and in greenhouse conditions before field evaluation (Brunck et al. 1990, Galiana et al. 1998).

Ultimately, it is also possible to select and obtain the most efficient N_2-fixing combinations between the genotype or tree provenance and the rhizobium or *Frankia* strain (Sougoufara et al. 1992). The mode of inoculation is also very important in trees, to maximize the early stages of Rhizobium colonization and to ensure a long-term persistence of the introduced selected strains in the field (Diem et al. 1988, Galiana et al. 1994, Galiana et al. 2002).

The use of molecular tools, such as DNA/DNA hybridization, PCR/RFLP or DNA sequencing, is essential to characterize the indigenous or introduced rhizobium strains, to define their taxonomic position (Zakhia and de Lajudie 2002) or for ecological studies on competition and survival of rhizobia in soils.

Agronomic Methods

Agronomists are able to improve the ANF in reducing the effect of limiting factors, more particularly through fertilization and management of N transfers.

Fertilization

In many situations, in particular for crops growing in alley cropping systems on soils of low fertility, it is necessary to apply a mineral complement of P and Ca to allow tree establishment and good nodule functioning, and ultimately the recycling of elements. The more rapidly the trees grow in the early stages of plantation, the faster the roots assimilate elements from deep horizons (Sanchez 1987).

Management of N Transfers (Restitution)

To ensure an optimal synchronization between the time of maximal mineralization of the prunings and nutritional needs of the cultivated crops, the pruning times should be planned accurately (Sanginga et al. 1988).

However, this is difficult to apply in practice since the kinetics of leaf or twig mineralization remains unknown so far in most species. The mineralization rate is known to be controlled by the activity of macro- and microfauna in soils (Anderson and Sinclair 1993). The restitution of the exported elements is an essential requirement for an efficient management of the fertility of cultivated soils (Ganry 1990).

CONCLUSION

To ensure the sustainability of any agricultural production, it is necessary to develop and implement methods capable of equilibrating the balance in soil elements (Ganry et al. 2001). With respect to N, this equilibrium can be reached through two ways: fertilization or BNF by cultivated plants and associated trees. In the current management conditions of agroforestry systems, the contribution of the trees to the equilibrium of soil N balance is generally insufficient. The development of new biotechnologies could improve BNF in the long term. Currently, some applicable methods and techniques are already available that can stimulate BNF, in particular the inoculation in the nursery and the vegetative propagation of clones with high N_2-fixing potential. However, these experimental techniques are far from being generalized in practice. The inoculation of tree seedlings in the nursery is very cheap in comparison with the inoculation of annual plants in the field: 1,000 to 2,000 trees ha^{-1} have to be inoculated *versus* 200,000 to 400,000 plants for annual crops.

Few economic studies have been reported about agroforestry systems. However, Palada et al. (1992) showed that an annual crop in an alley-cropping system with *L. leucocephala* was profitable with additional low fertilization (30 N-13 P-24 K per ha). It could be also assumed that the profit obtained from this culture system could even be higher if *L. leucocephala* was inoculated with performing rhizobium strains (Sanginga et al. 1988).

Lastly, the interest in planting N_2-fixing trees is increased when they are used as multipurpose species. This has been the case with some dry-zone Australian *Acacia* species that produce, in addition to wood, fodder with a high protein value for livestock or fruits and edible seeds (Thomson 1992).

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11

Architecture and Maps of the Chickpea Genome: a Basis for Understanding Plant-*Rhizobium* Interactions

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ABSTRACT

Chickpea is a high-protein grain legume crop of considerable nutritional and agronomical value with the potential to contribute substantially to biological nitrogen fixation, soil improvement and erosion prevention in arid and semiarid regions around the world. However, chickpea production is lagging far behind its potential, because the crop is susceptible to a whole series of biotic and abiotic stresses that decrease the efficiency of symbiotic nitrogen fixation and crop yield, and diminish its acceptance by the farmers. Recent years have seen considerable progress in our understanding of the general architecture of the chickpea genome and the genetics of factors that influence chickpea's agronomical value. Modern biotechnological tools such as fluorescent in-situ hybridization and DNA markers were applied to reveal the structure and evolution of the chickpea genome, to map genes responding to biotic and abiotic stresses, and to explore the synteny between chickpea and model plants. First efforts were made to learn about chickpea´s transcriptome, using genome-wide expression profiling techniques. Information about the genetics and transcriptomics of nodulation and nitrogen fixation in chickpea is scarce, due to low number of well-characterized nodulation mutants, non-existing nodule-specific EST libraries and other tools, demanding considerably more and intense research in this area. The transfer of knowledge from model plants and advanced crops together with high-throughput technologies will catalyze the analysis of the

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entire genome, transcriptome and proteome, and with it progress in this long neglected crop, adding substantially to its agricultural value.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a grain legume crop with a seed protein content of about 20%, especially suited for rainfed agriculture in cereal/legume intercropping systems of dry and semi-arid areas. Currently the crop is only a minor player on the world market, with a production of approximately 6.45 million metric tons in 2001 (FAOSTAT Agricultural Data 2001). On the Indian subcontinent, in West Asia and North Africa (WANA) and the Mediterranean basin, chickpea is mostly grown for local consumption, whereas in the Americas, Australia and Canada it gains growing importance for export. Like most other legumes, chickpea fixes atmospheric N_2 via symbiosis with bacteria of the genus *Rhizobium* (Caetano-Anollés and Gresshoff 1991a, Long 1996, Provorov et al. 2002). Symbiotic nitrogen fixation (SNF) itself, however, is not a major limiting factor for chickpea yield (Ali et al. 2002). Therefore, the complex interactions between the crop and rhizobia were not in focus. Instead, abiotic and biotic stresses such as drought and cold, together with limitations in the availability and utilization of trace elements (especially phosphorus), limit chickpea production and acceptance by farmers and are major constraints to SNF. Also, chickpea's most important fungal pathogens, *Fusarium oxysporum* f. sp. *ciceri* (Jiminéz-Diaz et al. 1993) and *Ascochyta rabiei* (Singh et al. 1992, Kaiser 1997) are major constraints to increased productivity (Saxena 1992)., In the Mediterranean basin, chickpea yield could be doubled by sowing in December to exploit winter rainfalls in this region and to allow for longer vegetative growth (Saxena 1992). Moreover, SNF increases by almost 250% under these conditions (RS Malhotra, personal communication). Nevertheless, farmers prefer the traditional spring-sowing, because cold and wet weather favors the spread of *Ascochyta* blight, which may cause complete crop loss by affecting all aerial parts of the plant (Kaiser 1997). Because of these biotic and abiotic stresses, worldwide chickpea production stagnates over decades at an average of 0.7 to 0.8 t ha^{-1}, though 4 to 5 t ha^{-1} could be obtained. It is for these reasons that chickpea breeding aims at developing high-yielding cultivars, combining long-lasting resistance against *Fusarium* wilt and *Ascochyta* blight with dehydration tolerance.

Biotechnological Tools to Assist the Chickpea Breeder

In recent years, biotechnology has developed techniques and tools to speed up and focus the time- and money-consuming process of developing improved varieties. DNA markers and dense genetic maps are especially extremely useful,

since they allow one to tag genes and follow their inheritance in segregating populations, so that breeders can predict the inheritance of a specific trait without field testing (reviews by Winter and Kahl 1995, Baum et al. 2000).

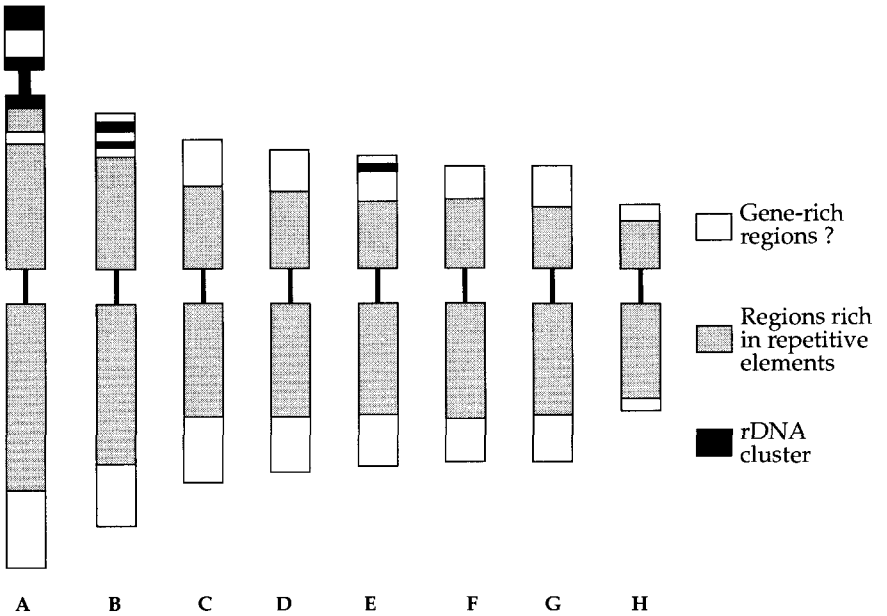
However, DNA markers certainly are not sufficient, since many traits, especially those controlled by polygenes, so-called quantitative trait loci (QTL, Young 1996), are too complex to be understood on the basis of markers only. Many QTL, such as those controlling the plant's reaction to abiotic stresses and its interaction with a wide spectrum of soil-living microorganisms including rhizobia and arbuscular mycorrhyza that are important for the performance of a particular genotype in the field, are complex and involve many metabolic pathways (Read et al. 2000, Provorov et al. 2002). Their manipulation requires a deeper understanding of the genome as well as the transcriptome and proteome, which can be analyzed by whole-genome expression profiling techniques such as cDNA and protein microarrays, or serial analysis of gene expression (SAGE), to mention only few. Developed for the study of human diseases and the analysis of model organisms, such techniques can now also be readily applied for chickpea improvement.

This paper reviews recent progress made in our understanding of the chickpea genome, and reports a first analysis of its transcriptome. It discusses options for chickpea SNF research to benefit from recent developments in model and advanced crop legumes. The paper is dedicated to many of our colleagues, since these achievements would not have been possible without the tight collaboration between the groups at ICARDA (Aleppo, Syria) and Frankfurt with Fred J Muehlbauer's group (Pullman, USA), and many other researchers throughout the world

THE GENERAL ARCHITECTURE OF THE CHICKPEA GENOME

Understanding the structure of a genome not only requires knowledge of the order of genes along the chromosomes, but also characterization and localization of its major components: repetitive elements. Repetitive sequences are ubiquitous, major constituents of higher plant genomes, and at least partly responsible for genome size and complexity. They vary in length between 1 and 10,000 bp and can be reiterated hundreds to several thousand times. The different repetitive elements are either widely dispersed, or clustered at only a few chromosomal sites. Well-defined functions are known only for a few repeat families such as telomeric or centromeric sequences, or 18S-5.8S-25S and 5S rRNA gene clusters encoding structural RNA components of ribosomes.

Two main types of repeats can be distinguished. **Tandem arrays** consist of contiguous stretches of repetitive units. They comprise rDNA gene clusters, micro- and mini-satellites and satellite DNA. The latter may account for several percent of a plant genome, whereas rRNA genes and mini- and



Source: Staginnus 1999, modified.

Figure 1. General architecture of the chickpea genome.

microsatellites are only of medium or low abundance. Usually, satellite DNA is located at a few distinct sites (Schmidt and Heslop-Harrison 1998). **Dispersed elements,** mostly pseudogenes, processed pseudogenes, orphons, transposons, retrotransposons or their remnants, are often interspersed with single copy sequences or other repetitive elements. Retroelements represent up to 50% of the genomic DNA in some plants (SanMiguel et al. 1996), translocate via RNA intermediates, and are usually dispersed along chromosome arms.

Major Repetitive Elements and their Distribution in the Chickpea Genome

The chickpea genome harbors at least three major families of repetitive elements. Two, *CaSat1* and *CaSat2*, are satellite families, the third, *CaRep1*, is a member of the *Ty3-gypsy* retrotransposon family (Staginnus et al. 1999).

Both **satellite families** display the typical organization of satellite repeats, with long tandem arrays of head-to-tail oriented repetitive units. With its 162-168bp long repeat units, the *CaSat1* family matches the unit length of many plant satellites, whereas repeats of the *CaSat2* family are only 100 bp in length (Staginnus et al. 1999). The satellite sequences constitute a considerable

part of the DAPI-stainable, pericentromeric heterochromatin, as shown by fluorescence *in situ* hybridization (FISH) on metaphase chromosomes (Staginnus et al. 1999). The majority of *CaSat1* probes locate to the centromeres of chromosomes A and B – close to the secondary constriction in the vicinity of the 18S-5.8S-25S rRNA gene blocks on A, and within the pericentric heterochromatic block on B (Staginnus et al. 1999). *CaSat2* elements are dominant components of the DAPI-positive pericentric heterochromatin of *all* chromosome pairs, including the centromeric regions. On chromosomes A and B, the clusters reside in close vicinity, but clearly separated from the major *CaSat1* sites. The high intensity of hybridization signals in metaphase and interphase nuclei suggests that *CaSat2* elements are the most abundant sequences in the chickpea genome.

The satellite repeats are useful taxonomic markers, because they are found solely in species closely related to chickpea. For example, the genome of *C. cuneatum*, formerly classified into section Monocicer together with chickpea, does not contain these elements, whereas *C. chorassanicum* (usually grouped into section Chamaecicer) does (Staginnus et al.1999), as also the perennial *C. anatolicum* (C Staginnus, unpublished). The presence or absence of such major structural genomic components is therefore highly informative characters for phylogenetic studies, and their absence in *C. cuneatum* excludes a direct common ancestor with the other species of the section Monocicer. The reclassification of this species is therefore necessary.

The third, highly abundant family of repetitive elements, *CaRep*, comprises sequences with homology to different parts of the *Ty3-gypsy* type **retrotransposon** *del1* from *Lilium henryi* (Smyth et al. 1989, Staginnus et al. 1999). The majority of the *CaRep* family members are clustered in the pericentric heterochromatin on all chromosomes. The uniform hybridization signals along the DAPI-positive heterochromatic blocks are only interrupted at the centromeric regions – probably consisting of *CaSat2* sequences. *CaSat1* elements on chromosomes A and B do not interfere with *CaRep* signals, but reside in the distal areas of the heterochromatin block, whereas *CaRep* elements are located adjacent to them in the proximal parts of the block. *CaRep* repeats are also absent from the secondary constriction harboring the 18S-5.8S-25S rRNA gene clusters on chromosome A. *CaRep* elements are not restricted to heterochromatin, but also reside in the gene-rich distal euchromatin of chromosome arms (Staginnus et al. 1999). In this respect, *CaRep* repeats are similar to those from other plants, where retrotransposon-derived sequences intermingle with plant genes (SanMiguel et al. 1996).

In addition to the three major repeat families, at least four non-tandemly arranged, dispersed repeat element families of middle to low abundance exist in the chickpea genome. All display homologies to transposable elements as e.g. *Ty-copia*-like elements, non-LTR retrotransposons and *En-Spm*-like transposons (Table 1). None of these repeat families is restricted to the genus

Cicer. The majority of these elements is located in the distal parts of the AT-rich heterochromatic blocks or in the euchromatin of several or all chromosomes, but are excluded from rRNA gene clusters and the highly repetitive satellite sequences.

As shown in Fig. 1, the localization of repetitive elements in the chickpea genome seems to be similar to that in the genome of the model legume *Medicago truncatula*. There, large portions of the chromosomes surrounding the centromeres are occupied by repetitive elements, and not much more than 100 Mbp of the whole genome is rich in genes (R Geurts, personal communication.). It is very likely that the gene-rich region of the chickpea genome is of similar size and is thus a promising target for genome sequencing. Using repetitive elements as probes for bacterial artificial chromosome (BAC) libraries could help to distinguish BACs from gene-rich and non-gene-rich regions.

MICROSATELLITES AND THEIR DISTRIBUTION IN THE CHICKPEA GENOME

The chickpea genome – like that of all other higher eukaryotes – additionally contains repetitive elements, so-called *microsatellites*, which have attracted much attention in recent years, since their variability makes them ideal markers for the identification of individuals and for genetic mapping.

Microsatellites (Litt and Luty 1989), also called simple sequence repeats (SSRs) or short tandem repeats (STRs), consist of reiterated sequence motifs of about 1 to 5 nucleotides such as $(A)_n$, $(AT)_n$, $(GA)_n$, $(CTT)_n$, $(TAGG)_n$ or $(TTCGG)_n$. Like satellite DNA, microsatellites are organized in more or less perfect tandem arrays of few to hundreds or even thousands of repeat units. A key feature of microsatellites is a strong tendency to change their overall length leading to variable numbers of tandem repeats (VNTR), that result in simple sequence length polymorphisms (SSLPs). SSRs are abundant and usually evenly dispersed throughout plant genomes. High levels of polymorphism, abundance, and ubiquitous occurrence recommend SSRs as exceptionally useful molecular markers (see reviews by Gupta and Varshney 2000, Winter et al. 2002a).

Molecular Markers for Genetic Mapping of the Chickpea Genome

Chickpea is probably of monophyletic origin and arose by selection from its ancestor C. *reticulatum* (Zohary and Hopf 1993, Zohary 1999, Lev-Yadun et al. 2000). Genetic bottlenecks imposed during domestication and breeding practices narrowed the genetic base of the obligatory self-pollinating crop as compared to its wild founder species. Therefore, the many variations of qualitative and quantitative traits in chickpea are astonishing. However,

Table 1. Repetitive sequence families in the chickpea genome.

Repetitive element	Type	Abundance
CaSat1	Satellite DNA	high
CaSat2	Satellite DNA	high
CaRep	*Ty3-gypsy*-like LTR retroelement	high
CaTy	*Ty1-copia*-like LTR retroelement	middle
CaDis	Retroelement	middle
CaLin	LINE-like non-LTR retrotransposon	low-middle
CaEn/Spm	*En-Spm*-like transposable element	low-middle
Microsatellite	Mono-, di-, tri-, tetra- and pentanucleotide repeats	high

Source: Staginnus et al. 1999, 2001

they are probably not a result of large-scale interspecific genetic variation, but instead caused by mutations in single genes.

Linkages between several of these physiological and morphological characters and between biochemical and DNA markers have been reported (Simon and Muehlbauer 1997). However, mapping was considerably hampered by the monotony of the chickpea genome. To circumvent the problem of low genetic variability within the cultivated species, all published molecular marker-based maps were formerly derived from interspecies crosses between chickpea and *C. reticulatum* or *C. echinospermum*, respectively (review by Winter et al. 2002b).

Some repetitive elements are useful: microsatellite-based markers

Notwithstanding the overall monotony of the chickpea genome, Weising et al. (1989, 1992, 1995, 1998, Sharma et al. 1995 a, b) found considerable variation at the intra- and interspecific level by RFLP analysis with SSR-specific oligonucleotides. Relying on hybridization of end-labeled oligonucleotide probes complementary to SSR motifs (e.g. $[GATA]_4$) to restricted and electrophoretically separated genomic DNA, the so-called DNA fingerprinting technique generates highly informative, multiple banding patterns (multilocus RFLP fingerprints), that allow even the differentiation of individuals.

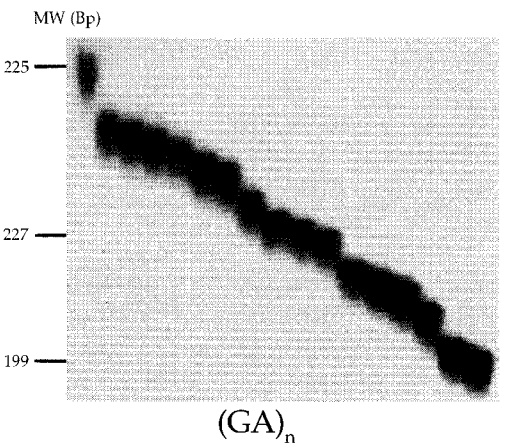
Other SSR-based methods use SSR-complementary oligonucleotides as PCR primers, either alone or in combination with arbitrary or specific primers, to amplify distinct regions of genomic DNA (see Gupta and Varshney 2000). Particularly microsatellite-primed (MP)-PCR (Meyer et al. 1993, Gupta et al. 1994), anchored microsatellite-primed (AMP)-PCR (Zietkiewicz et al. 1994), random amplified microsatellite polymorphism (RAMP, Wu et al. 1994), selective amplification of microsatellite polymorphic loci (SAMPL, Vogel and Scolnik 1997), random amplified microsatellite polymorphism (RAMPO, Richardson et al. 1995), and retrotransposon-microsatellite amplified polymorphisms (REMAP, Kalendar et al. 1999) were tested. Further, methods

not directly targeting at VNTR-type polymorphisms, such as DNA amplification fingerprinting (DAF, Caetano-Anollés et al. 1991b) and amplified fragment length polymorphism (AFLP, Vos et al. 1995) were also studied.

The suitability of these methods for the detection of genetic variability between chickpea accessions and *Cicer* species varies considerably. Almost all of them are useful for mapping interspecific populations, but rarely detect polymorphisms between chickpea accessions. Moreover, all these methods provide dominant markers, that normally cannot be transferred from one population to another, and do not reliably detect heterozygotes (for more detail see Winter et al. 2002b).

Locus-specific amplification of microsatellites: STMS markers

However, highly polymorphic, co-dominant markers can be generated by locus-specific amplification of SSRs with specifically designed primers directed towards their flanking sequences (Litt and Luty 1989, Weber and May 1989). The resulting amplification products often exhibit considerable length variation among different individuals or populations of the same species, mostly due to variable numbers of tandem repeats within the SSR (Fig. 2). These sequence-tagged microsatellite site (STMS, Beckmann and Soller 1990) markers are the tools of choice for nearly every organism. They are single-locus, co-dominant, easy-to-use and reliable markers with high polymorphic information content possessing the potential for automated, non-radioactive detection.



Source: Hüttel et al. 1999, modified.

Figure 2. Allelic variation of a $(GA)_n$-containing STMS locus in various accessions of chickpea from different geographic origins.

In spite of the many advantages of STMS, high costs of cloning, sequencing and primer synthesis, and the use of radioisotopes and sequencing gels to detect the amplified SSRs precluded their large-scale generation and application for long. These drawbacks are the reason that to date only 270 chickpea STMS have been generated (Hüttel et al. 1999, Winter et al. 1999), which are now used for mapping throughout the world (e.g. Tekeoglu et al. 2002, Cho et al. 2002, Udupa and Baum 2003).

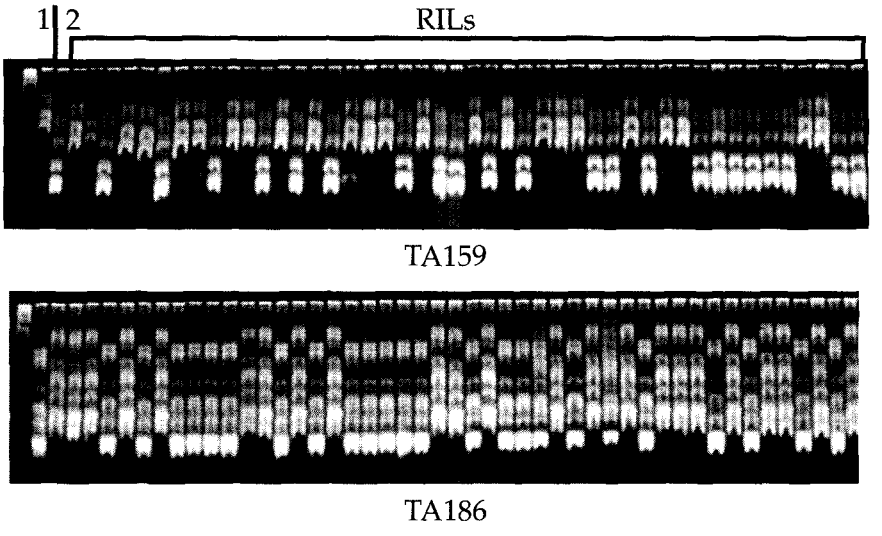


Figure 3. Segregation of 2 STMS markers in recombinant inbred lines of chickpea. Photo of an ethidium bromide-stained 6% native polyacrylamide gel.

GENOME MAPPING IN CHICKPEA

The availability of STMS markers and several populations of recombinant inbred lines (RILs, Burr et al. 1988) from interspecific crosses allowed the generation of a first co-dominant DNA marker map (Winter et al. 1999), which served as a backbone for subsequent maps of higher density. This map contained 112 STMS segregating in a population of 90 RILs from an interspecies cross between *C. reticulatum* accession P.I. 489777 and the *Fusarium* wilt resistant cultivar ICC 4958. Markers were located in 11 linkage groups covering 613 cM.

Building on this STMS skeleton map, we used 130 RILs from the above cross to extend the map. Mapping in RIL populations has the advantage that, contrary to F_2 populations, dominant and co-dominant markers have similar information content. Therefore, the integration of economic, dominant markers such as RAPDs, DAFs, AFLPs and ISSRs into a co-dominant STMS

framework was possible (Winter et al. 2000). Since the parental line ICC4958 is resistant to races 0, 4, and 5 of *Fusarium oxysporum*, and *Cicer reticulatum* accession P.I. 489777 is susceptible, the segregation of the underlying resistance loci *foc0*, *foc4* and *foc5* could be followed. Besides these resistance loci, 351 other markers were mapped. At a LOD-score of 4.0, 303 markers covered 2077.9 cM in 8 large and 8 small linkage groups at an average distance of 6.8 cM between markers. Fifty-one markers (14.4%) were unlinked. The large linkage groups probably represent the eight chickpea chromosomes. This has been proven for the smallest linkage group 8, which represents the smallest chromosome H as demonstrated by amplification of flow-sorted chromosomes with STMS markers (Vláèilová et al. 2003). Clustering of

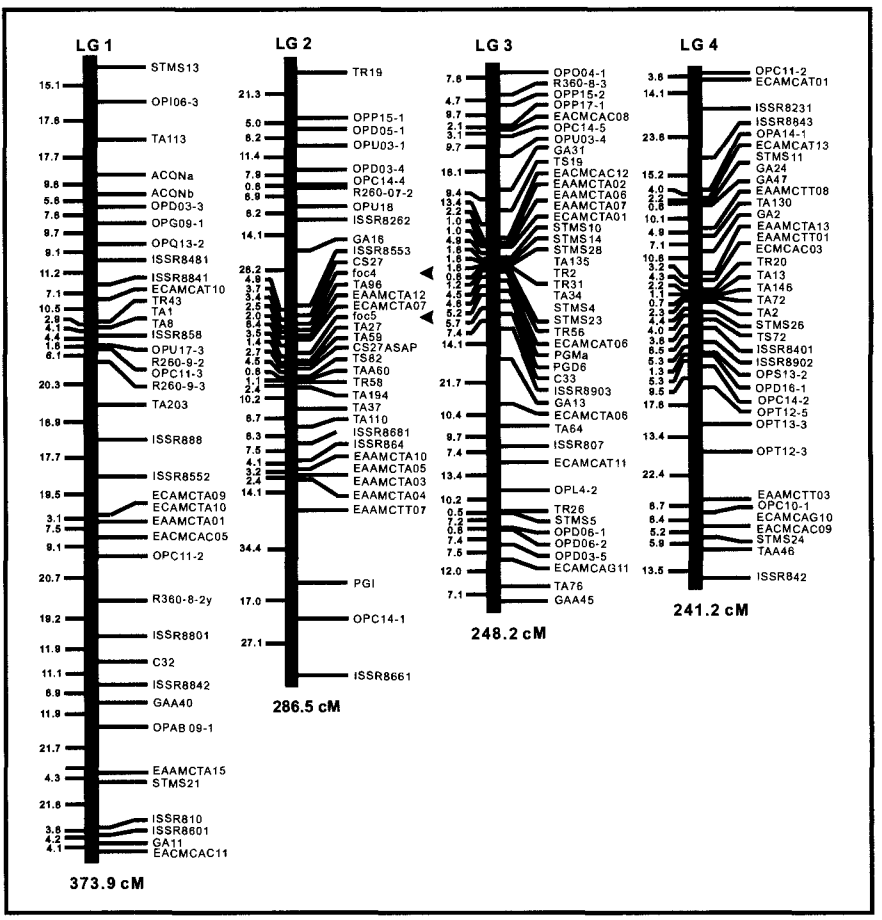


Figure 4. Part of the genetic map of the chickpea genome derived from segregation analysis of markers in RILs from the cross ICC4958 x C. reticulatum P.I.489777.

markers in central regions of linkage groups was observed. Markers of the same class (except ISSR and RAPD markers) tended to generate sub-clusters. The *foc4* and *foc5* loci mapped to linkage group 2 together with STMS and an ASAP marker previously linked to the locus conferring resistance to *Fusarium* race 1 (*foc1*, Ratnaparkhe et al. 1998). Clustering of wilt resistance genes around this locus is therefore possible. Significant deviation from the expected 1:1 segregation ratio was observed for 136 markers (38.4%, $P<0.05$), and in 68% of the cases segregation was biased towards the wild progenitor. Segregation distortion was similar for all marker types except ISSRs, that showed only 28.5% aberrant segregation (Winter et al. 2000).

GENETICS AND FUNCTIONAL GENOMICS OF SYMBIOTIC NITROGEN FIXATION IN CHICKPEA: ENTERING TERRA INCOGNITA

A survey of recent literature reveals an unprecedented burst of knowledge about the physiological and genetic processes underlying legume-*Rhizobia* symbiosis, nodule formation and N_2 fixation. Most of this information comes from studies in the model legumes *Medicago truncatula* and *Lotus japonicus*, profiting from large numbers of either naturally-occurring or induced mutants that help to identify genes, signaling processes and metabolic pathways involved in SNF (Searle et al. 2003, Krusell et al. 2002, Nishimura et al. 2002, Oldroyd and Long 2003, Penmetsa et al. 2003, reviews in Schauser et al. 1998, Kawaguchi et al. 2002). Also in chickpea, hyper-, non- or low nodulation mutants have been detected (see recent review by Bathia et al. 2001) and crosses between low and good nodulating accessions were performed (Rupela and Saxena 1987, Singh et al. 1992, Davis et al. 1992). However, mapping genes involved in SNF and other traits was hampered by a lack of polymorphism in the chickpea genome, restricting the use of DNA and other markers. Since highly polymorphic STMS markers are now available and will be increased in number, the factors most important for efficient SNF could and should be mapped also in chickpea.

Progress is becoming extremely rapid in the field of functional genomics. Again, the model legumes are way ahead. Large collections of ESTs are available from nodulated roots of *Medicago* (Gyorgyey et al. 2000) and several nodulation genes have been mapped (Ane et al. 2002). Especially cDNA arrays demonstrate their usefulness to detect transcripts involved in SNF. In *Lotus*, for example, Colebatch et al. (2002) used an array of 2,304 cDNA clones derived from N_2-fixing nodules to detect differences in relative transcript abundance between nodules and uninfected roots. Transcripts of 83 different genes were found more abundant in nodules than in roots. More than 50 of these had never before been recognized as induced in nodules in any species. Expression of 36 genes was detected in nodules, but not in roots. Several

known nodulin genes were among the nodule-induced genes. However, not only in model legumes with small genomes is whole-genome transcript profiling possible. Using a cDNA microarray for soybean that contained approximately 4,100 Unigene ESTs derived from soybean axenic roots, Maguire et al. (2002) demonstrated the utility and reliability of this technology for functional genomics also in a crop with a large and complex genome by verifying microarray-derived results with both quantitative real-time RT-PCR and Northern blot analysis. There was a linear correlation ($r^2 = 0.99$, over 5 orders of magnitude) between microarray and quantitative real-time (RT)-PCR data. In this study several ESTs showed high levels (>50 fold) of differential expression in either root or shoot tissue.

In spite of its importance also for an agronomic performance of chickpea, SNF research in this crop is lagging far behind the models and other crops, e.g. pea or alfalfa. This is also stressed in a survey of recent literature available at the National Library of Medicine (PubMed at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&DB=PubMed). Using 'nodulation' as search term delivers almost 1000 entries. However, searching for 'chickpea nodulation' results in 4 hits dealing with agronomic factors affecting SNF (Rao et al. 2002, Sindhu and Dadarwal 2001a,b, Kyei-Boahen et al. 2001). Also, only 8-gamma ray-induced and 13 spontaneous chickpea SNF mutants are listed in a recent review by Bathia et al. (2001), whereas the same paper counts almost 30 well-characterized mutants for soybean and 70 for pea.

The situation is even more discouraging if symbiosis between chickpea and mycorrhiza is considered. Although it is well known that mycorrhizal fungi can significantly improve the solubility and uptake of nutrients by plant roots and stabilize the water balance, especially in marginal soils (see for example Joner 2000, Fagbola et al. 2001), almost nothing is known about the interaction of chickpea with beneficial fungi. Also, here, much can be learned from advanced crops (Provorov et al. 2002).

Since lots of resources are now available from the model legumes and advanced crops such as pea and soybean, the time has come to dig these gold mines for SNF research in chickpea. Transfer of knowledge from the models to the chickpea crop is a multifaceted process, but can be accomplished if the necessary funds are available. Single steps include:

1) systematic analysis of existing chickpea SNF mutants
2) large-scale generation and identification of new chickpea SNF mutants by ethyl methane sulfonate (EMS) mutagenesis and physiological tests
3) **T**argeting **I**nduced **L**ocal **L**esions **I**n **G**enomes (TILLING, McCallum et al. 2000) analysis of mutations focusing on known *NOD* and *ENOD* cDNAs of model plants

4) generation, testing and genetic mapping of inter-and intraspecific populations from crosses between SNF mutants and SNF high-performance lines using these genes and STMS as markers
5) functional genomics of mutant, normal and high-performance SNF lines by hybridization of their nodulated and non-nodulated root cDNAs to cDNA-chips available from the model legumes

In spite of the gloomy future for improving SNF in chickpea using biotechnological approaches, researchers must keep in mind that besides biotic stresses, crops are also subject to environmental stresses such as nutritional constraints and drought and cold. These significantly reduce the ability to fix N_2 even of superior varieties, and thus impose major constraints on their productivity. The development of stress-tolerant germplasm is therefore necessary to overcome these limitations and increase productivity in the face of an expanding human population and ever-growing demand for food legumes. Traditional approaches to improving germplasm are limited by the complexity of drought and cold tolerance traits, low genetic variance of yield components under stress conditions, and inefficient selection techniques.

The first step towards categorizing genetically complex abiotic stress responses is the discovery of stress-responsive genes by large-scale partial sequencing of randomly selected cDNA clones or expressed sequence tags (ESTs). Extensive EST collections already exist for the model plants *Arabidopsis thaliana*, *Lotus japonicus*, and *Medicago truncatula*, and major crops like rice, maize, soybean and others (dbEST section of GenBank). These sequencing efforts have generated collections in which more than half of the total gene complement of crop plants is represented. The data base collections are, however, biased due to their sampling approach. Functional genomics solves this problem by linking the most important stress-related transcriptional responses of the plant to its performance in abiotic stress environments.

CHICKPEA FUNCTIONAL GENOMICS

With the recent development of microarray technologies, the fast and reliable quantification of specific mRNA levels is possible, reflecting the physiological potential of the plant. Consequently, we aimed at the generation of a DNA chip in order to determine the transcriptional activity in response to abiotic stresses of many genes of legumes in general. This genome-wide screening allows prediction of the physiological potential of breeding material, localization of stress-responsive genes and mapping of quantitative differences in gene expression levels in segregating populations under stress.

In a first attempt to test the suitability of microarrays for the analysis of stress responses in chickpea, we selected sequences of stress-responsive

cDNAs from *Lotus japonicus* and determined domains homologous to sequences from other plants. Primers targeted at these domains were used to amplify the respective sequences from chickpea or *Lotus* cDNA, respectively. Several chickpea genes responding to biotic stress were known from the work of Barz and colleagues (Ichinose et al. 2000), and some of these were included. Besides a set of housekeeping genes (GAPDH, ubiquitin, actin, etc.) as controls, cDNAs encoding proteins involved in different responses to abiotic and biotic stresses were bound to the chip. These included cDNAs specifically induced by pathogens (e.g. encoding an *AvrRpt2*-inducible protein), or genes for components of defense response (e.g. *hydroxynitril-lyase*), and parts of potential resistance genes from chickpea (Huettel et al. 2002). Other genes responding to oxidative stress (e.g. *peroxidase, glutathione-S-transferase*), or involved in metabolism of aromatic compounds (e.g. *chalcone synthase, cinnamoyl-CoA- reductase*), fatty acid signaling and metabolism (e.g. *lipoxygenase*) and signal transduction (e.g. receptor protein kinases) were also spotted. The chip is available from GenXPro, Frankfurt, Germany, at low cost.

For a first test, the chip, containing 151 cDNAs (107 from *Lotus japonicus* and 44 from chickpea), each spotted in triplicate, was hybridized to cDNA from untreated and wounded chickpea leaves labeled with fluorochromes cy3 and cy5, respectively (Table 2). Of the 150 cDNAs on the chip, 12 were induced at least twofold. *Ubiquitin*, thought to be constitutively expressed, belonged to this group. Several genes were significantly repressed, involving defense-related genes such as *gluconase* and all 3 *chitinases*, which were down regulated more than 3 fold.

These experiments proved that stress-responsive DNA fragments may principally be obtained by targeting conserved domains of stress-responsive genes from different related legumes and amplification of the respective sequences (Pinkert 2002).

However, species-specific and even accession-specific responses may exist, that cannot be detected that way. To explore the specific stress-related transcriptome of chickpea we use an additional technique that not only presents an overview of almost all of the chickpea transcriptome but also enables evaluation of results obtained by microarrays. This technique, SAGE

Table 2. Examples of genes significantly induced or repressed in wounded chickpea leaves as compared to untreated controls. The species from which the spotted cDNA was derived is also given (L=*Lotus japonicus*, C=chickpea).

Genes induced by wounding	Fold induction over control
Peroxidase (C)	8.85
NBS-LRR5 (L)	5.30
Peroxidase (L)	4.87
Cysteine proteinase (L)	2.50
Ubiquitin (L)	2.30

(Velculescu et al. 1995), is an efficient, reliable and comprehensive technique for the quantification of gene expression. It is based on two fundamental principles: (1) A short sequence of 9 to 11 bp, a so-called tag, contains sufficient information to uniquely identify a transcript, and (2) concatenation of tags in a serial fashion allows for increased efficiency in a sequence-based analysis. These properties render SAGE 20 to 30 times more efficient in gene expression screening than EST sequencing (Matsumura et al. 1999).

In a first attempt to apply the SAGE technique to whole-genome expression profiling of chickpea, cDNAs from control and wounded roots were analyzed. A total of 787 concatemers were sequenced representing 1590 cDNAs from control and 2124 cDNAs from wounded roots, respectively. There were impressive differences in the expression of certain genes, especially those that were up-regulated. A considerable number of genes were also down-regulated (data not shown).

However, the drawback of SAGE is that the obtained sequences are often located at the species- and gene specific 3'-untranslated regions of mRNA. Therefore, and in the absence of a large EST database, it is presently not possible to identify the genes that are represented by the 10 bp tags by data mining alone. However, the first SAGE study in plants by Matsumura et al. (1999) revealed that more than 75% of differentially expressed genes in abiotically stressed rice plants were neither published nor available in data bases. An extended EST sequence database is therefore required also for non-model plants like chickpea.

OUTLOOK

The past few years have seen impressive progress in our knowledge of chickpea genomics, and first attempts to understand the chickpea transcriptome are under way. Moreover, international research in model legumes has considerably expanded our insight into the complex mechanisms that govern agronomically important traits such as stress tolerances and plant-microbe interactions. For plant breeders it will be one of the challenges of the next decade to transfer this knowledge to crop plants and exploit it for the benefit of an ever-growing world population.

However, many questions cannot be solved with model plants since many crops have unique properties developed during domestication. Also, tools to increase the efficiency of breeding, such as molecular markers developed for model plants, cannot easily be transferred to crops since these often have streamlined genomes where such markers are not informative. Therefore, crop-specific markers have to be developed that specifically target agronomically important loci and are highly informative, robust, easy-to use and cheap to satisfy the needs of the breeder.

MARKER-ASSISTED BREEDING: TOWARDS APPLICATION OF MARKER TECHNOLOGY IN CHICKPEA

Formerly, all published genetic maps of the chickpea genome were based on interspecies crosses between the cultigen and the wild relative *C. reticulatum*. Also, mapping of resistance loci for chickpea's most important fungal pathogens relied on such crosses. The low level of genetic polymorphism between chickpea accessions dictated these restrictions. However, chickpea breeding is based on intraspecific crosses. Today, the availability of STMS markers (Hüttel et al. 1999, Winter et al. 1999) allows the generation of intraspecific maps and the tagging of pathogen resistance (Flandez-Galvez et al. 2003, Udupa and Baum 2003) or double podding loci affecting chickpea yield (Cho et al. 2002). This demonstrates that the present marker technologies can deal with the monotony of the chickpea genome and support routine breeding work with simple, fast and cheap allelic tests. The STMS marker technology is especially suitable, since (1) STMS loci are sufficiently variable to detect differences even between closely related chickpea accessions and (2) the technique has the potential for high-throughput screening and automation.

However, the successful application of marker technologies requires: (1) a dense intra-species map linking traits of agronomic importance such as yield and flowering time (Kumar and van Rheenen 2000, Kumar et al. 2000a,b, Or et al. 1999), tolerances for the most important stresses (cold and drought), resistances against pests, and superior performance in SNF to highly polymorphic, co-dominant markers in sufficiently close vicinity to allow marker-assisted selection also in offspring from intra-species crosses. (2) for routine applications, the tailoring of STMS markers in combination with high-throughput screening techniques to speed up and simplify the application of marker technology. Successful optimization of STMS markers for multiplex PCR and electrophoresis has been reported for soybean, where up to 8 STMS markers can be amplified in a single PCR reaction and separated in a single lane of a sequencing gel. More than 70 of these tailored markers cover the whole genome (Narvel et al. 2000). However, this advancement built on the availability of more than 600 STMS markers for soybean (Cregan et al. 1999). In chickpea, the number of STMS markers is still too low for such applications. Consequently, a much larger set of STMS markers will have to be developed. This is not only true for chickpea but also for other crops with similar problems. In the past, the necessary investments were unachievable for minor crops. Today, advanced technologies that are also commercially available (as from GenXPro, Frankfurt, Germany) could make the benefits of advanced marker technologies available also for less important crops.

ELUCIDATING COMPLEX MECHANISMS OF CROP METABOLISM USING DNA MICROARRAYS: IMPLICATIONS FOR BREEDING AND IMPROVED SNF IN CHICKPEA

Since cDNA microarrays allow the analysis of the entire transcriptome at a time with relatively little effort, they are ideally suited for the selection of superior genotypes, given that the genes indicative for the trait in question are spotted. As our experiments with heterologous hybridizations of *Lotus*-derived cDNAs with chickpea cDNAs show, it is not even necessary to develop a chip for a specific species. Instead, legume-wide expression profiles can be obtained with cDNAs from model plants like *Lotus* and *Medicago*, and observations made there can immediately be exploited for leguminous crops. However, results from models cannot uncritically be transferred to crops. For the breeder the availability of DNA chips that are indicative of the performance of a plant under different environmental conditions has the advantage that the different metabolic pathways underlying a phenotype can now be resolved into their components, and desired ones can be combined in superior cultivars. Also, germplasm banks can be searched for transcriptionally highly diverged genotypes that may well be phenotypically similar. The spectrum of criteria for the selection of core collections could therefore be extended and the exploitation of the vast collections improved. Especially, SNF- microarrays will do a good job, since they allow monitoring of the reaction of a specific genotype of a crop to a particular strain of *Rhizobium* under different environmental conditions including nutritional constraints and stresses. This will enable the selection of the most promising plant-microbe combinations for a particular environment and also breeding for it.

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12

FABAMED Interdisciplinary Strategy to Improve Symbiotic Nitrogen Fixation of Legumes in the Mediterranean Basin

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ABSTRACT

FABAMED (Fixation de l'Azote dans le BAssin MEDiterranéen) is a cooperative research group of agronomists, plant breeders, microbiologists, physiologists and molecular biologists. It was created in 1995 with the goal of improving the symbiotic nitrogen fixation (SNF) and N_2-dependent growth of legumes under pedoclimatic limitations of the Mediterranean Basin. The INCO (INternational COoperation) project, FYSAME (nitrogen Fixation and Yield of grain legumes in SAlinized MEditerranean areas), financed by the European Union, illustrates this interdisciplinary strategy for moderate salinity adaptation of symbiotic chickpea and common bean. Agronomic surveys in reference production areas showed large spatial and temporal variations in plant nodulation and growth, and in utilization efficiency of the rhizobial symbiosis associated with large rhizobial diversity, including new bean-nodulating species. Macrosymbiont diversity in SNF and tolerance to NaCl was found with both common bean and chickpea. However, the contrasts in efficiency and tolerance between plant genotypes could be altered by specific interactions with some native rhizobia. Therefore, variations in soil rhizobial population, in addition to agronomic practices and environmental constraints, may have contributed to erratic results observed in field inoculation trials. At the mechanistic level, nodule C and N metabolisms, and abscisic acid content, was measured and related with nodule conductance to O_2 diffusion. The regulation of the latter in the nodule cortex was addressed by in-situ hybridization of candidate genes, namely carbonic anhydrase and aquaporin, on nodule section.

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INTRODUCTION

Legume-rhizobia symbiosis should be a priority research area for developing countries because of its ability to support production of protein-rich seeds and fodder by fixing nitrogen, and its beneficial effect on productivity of cereals and other crops in agricultural rotations. However, in the Mediterranean basin more than 40% of soils are saline, adversely affecting legume productivity. Draining salinized soils or irrigating them with good quality water from remote sources are extremely costly measures. Therefore, a collaborative research project was designed with the general objective of applying some of the spectacular recent advances in methodologies and basic knowledge on symbiotic nitrogen fixation (SNF) with the following specific objectives: (1) to select common bean (*Phaseolus vulgaris*) as a model legume and its native specific rhizobia for SNF potential and expression under NaCl salinity; (2) to assess yields in soils irrigated with water varying in salinity in North Africa and South Europe; and (3) to improve the understanding of biochemical mechanisms involved in SNF tolerance to salinity.

FABAMED addressed the following tasks:

1. To characterize the project reference zones for prospecting and further assessment of plant cultivars and *Rhizobium* strains through an initial agronomic survey on soil parameters and nodulation-N nutrition of the project legume(s).
2. To evaluate interactions between selected symbioses and the pedoclimatic environment by multilocal-pluriannual field inoculation trials in soils identified as relevant to the project according to the initial agronomic survey.
3. To screen for SNF potential and tolerance to environmental limitations among traditional or introduced legume genotypes and their specific native rhizobia.
4. To investigate intraspecificity for nodulation or nodule function by cross inoculation trials between relevant micro- and macrosymbionts with and without the locally determinant environmental limitation of the above agronomic approach (drought, salinity, phosphorus or iron deficiencies, aluminium or manganese toxicities).
5. To define parameters and controlled conditions for optimal screening of SNF potential and tolerance to the determinant limitation, to search for SNF-related mechanisms that are affected by the determinant limitation at the plant and cell levels.
6. To utilize molecular biology tools to characterize the genetic diversity of microsymbionts, to link with the molecular characterization of tolerant

and sensitive macrosymbionts, and to tag genetic determinants of SNF potential and tolerance to environmental limitations.

The project FYSAME (nitrogen Fixation and Yield of grain legumes in SAlinized MEditerranean areas) was multidisciplinary in nature including agronomists, microbiologists, plant breeders, physiologists and molecular biologists from Algeria, France, Morocco, Spain and Tunisia, with the following aims:

1. To survey salinized zones for collecting local cultivars and rhizobia, and to assess selected symbioses through field trials;
2. To screen macrosymbionts for SNF tolerance to salinity, and to perform cross-inoculation trials for microsymbiont infraspecificity with and without salinity;
3. To define optimal procedures for the above screenings, and to investigate cellular and molecular SNF mechanisms associated with salt tolerance, through comparative studies of sensitive *versus* tolerant symbioses.
4. To characterize the genetic diversity of microsymbionts, and tag the genetic determinants of macrosymbiont tolerance, for further investigation of genes involved in SNF tolerance to salinity.

The objective of this paper is to review FYSAME's most applied achievements, as an illustration of FABAMED research strategy, and address subsequent prospects and limits.

SPATIAL AND TEMPORAL VARIATION IN BEAN NODULATION

Nodulation surveys in Lauragais (France) as a control reference area showed large variation in nodulation from mean field values of less than 5 to more than 50 nodules per plant at flowering stage. In addition, large variations in nodule number and mass per plant were observed over years under similar developmental stages and soils (Drevon et al. 2003). In the Medjerda valley (Tunisia), the nodulation of common bean was low, especially at the sites of Beja and Jendouba where nodules could not be detected at either early vegetative or at flowering stages (Sifi 2003). In Bizerte and in Cap Bon in northern Tunisia, mean nodulation varied between 0.1 and 2.3 nodules pl^{-1}, although nodules disappeared before flowering in many fields where they were observed at early vegetative stage (Sifi 2002). In Morocco, nodulation was lower in Loukos than in Ain Atiq (Aurag et al. 2002).

From survey stations where N-fertilization improved the yield, the data of shoot biomass was plotted as a function of nodulation. The slope of this regression curve was used as an indicator of the efficiency of the utilization of the symbiotic nodules for plant growth. A high slope of the regression curve can be interpreted as high efficiency of use of the symbiotic N supply to

plant-growth and adequate complementarity between both sources of N, i.e. atmosphere and soil. This estimate varied between stations within an area, and between areas and years (Drevon 2001). Differences in its value might be due to variation in the symbiotic potential of the host-legume and/or the microsymbiont, and their interactions with environmental factors.

DIVERSITY OF NATIVE RHIZOBIA NODULATING THE COMMON BEAN

From the 300 isolates of the Tunisian collection, the 8 following taxons have been identified so far: *Rhizobium gallicum*, *R. etli*, *R. leguminosarum* bv *phaseoli*, *R. giardinii*, *Sinorhizonium fredii*, *S. meliloti*, *S. medicae* and 'pseudo-*Agrobacterium*' (Mahmdi et al. 1999). This large diversity has also been revealed by serological studies with 62% of isolates being in 19 different serogroups (Fekki et al. 2003). In addition, the structure of rhizobial populations differed significantly between the Medjerda valley, the Cap Bon and the semi-arid south of Tunisia. In Morocco, so far two isolates were identified as *R. leguminosarum* bv *phaseoli*, four as 'pseudo-*Agrobacterium*', and six as *R. tropici* B (Boumouch et al. 2001).

Species of the *Rhizobiaceae* family of bacteria, such as *R. gallicum* and *R. giardini*, were previously proposed as new species from French soils in addition to *R. etli* and *R. leguminosarum* bv *phaseoli*, which are commonly found in other parts of the world outside Latin America in soils where common bean has been grown for millennia (Amarger et al. 1997). In contrast, the *Sinorhizonium* spp. nodulating *P. vulgaris* were new in the soils of the Mediterranean basin. *R. tropici* B was previously found in soils of tropical Latin America and Africa. This needs further exploration since this species was found neither in France nor in Tunisia, as confirmed by serological studies. The isolation of *Agrobacterium spp.* from common bean nodules agrees with a previous report on *Acacia* spp by de Lajudie et al. (1999).

Nineteen rhizobia from Morocco and 30 from Tunisia appeared to be at least as efficient, or more efficient than *R. tropici* B CIAT899 in symbiosis with the local cultivar Coco. A large variability was found in the tolerance of native rhizobia to salinity in free-living culture (Aouani et al. 1998, Aurag et al. 2003). In addition, mutants with altered sensitivity to salinity were obtained from *R. etli* and *R. tropici* (Ben Abdelkhalek et al. 2003).

SELECTION OF SYMBIOTIC *P. VULGARIS*

Fourteen lines of *P. vulgaris* were selected as superior to Coco in Mateur (Tunisia) during the 3 years of observation although the difference varied between 1996 (a dry year), 1997 (normal), and 1998 (humid). In production areas of the Medjerda Valley in 1999, the lines DOR585, SVM29-21, Flamingo,

KID53, Ruddy, CAN74, BRB17 and WAF147 expressed mean yields higher than the 1.3 t ha^{-1} of Coco (Trabelsi 2003). Their nodulation with local rhizobia varied between 0 and 27 nodules pl^{-1} in KID53 and BRB17, respectively, whereas Coco harbored a significantly intermediate mean of 11 nod pl^{-1}. However, although these lines would respond to the farmer's demand for pod production, none had the seed characteristics to substitute Coco for the white grain local market. In Lauragais, variability in yield was found among 27 white-seed lines, with Diego producing more grain than the 2.5 T ha^{-1} of the local cultivar Linex (Rey-Poiroux et al. 2003). Nodulation varied between 5 nodules pl^{-1} for T815 to 50 nodules pl^{-1} for Diego.

The sensitivity of the above lines to moderate salinity was tested in the glasshouse with N nutrition depending on either the rhizobial symbiosis with *R. tropici* CIAT899, or the supply of mineral N (Saadallah et al. 2001a). For all lines tested, the nodulation was much higher in glasshouse than in fields. The amount of fixed-N was the parameter most affected. The plant growth was less restricted by the salt treatment with mineral N than with N_2. Coco was more sensitive than BAT477, DOR364, DOR585 and Flamingo, whereas BRB17, ABA16 and Dark were the most sensitive (Boughribil et al. 2003, Saadallah et al. 2001b). It was verified that these contrasts were not a consequence of any difference in seedling vigor. Salinity inhibited not only the nodulation process but also the nodule growth (except in DOR585) and the nodule function.

The higher sensitivity of Coco compared to BAT477 was associated with higher leaf content of Na^+ and Cl^-, and higher root content of Na^+ (Saadallah et al. 2001b). The latter was much higher than that of leaves, which agrees with the known exclusive behaviour of common bean. The nodule content of Na^+ was higher in BAT477 and Dark than in Coco. However, it was much lower than the nodule content in Cl^-. The latter did not differ significantly between contrasting lines. Nodule P content was not affected by salinity. A split-root experiment confirmed that the sensitivity of symbiotic common bean to NaCl was not due to toxic effects of Na^+ or Cl^- accumulation in nodules or leaves, but most probably to alteration of other nutrient acquisition (Lachaal et al. 2003). Though there was no significant difference in K^+ content for any organs in these studies, the higher sensitivity of symbiotic *versus* nitrate-fed plants was associated with an excessive accumulation of Cl^- in leaves and overloading of nodules with toxic Na^+ and Cl^- (Lachaal et al. 2003).

INTERACTION OF NATIVE RHIZOBIA WITH SELECTED BEAN LINES

Coco, BAT477, Flamingo and NAG310, and the rhizobia CIAT899, 12a3 (*R. etli*), and 1a6 (*S. fredii*) were selected from initial cross-inoculation trials with the eight lines and four local rhizobia from Tunisia, for further comparison in sand or hydroaeroponic growing conditions. The type of

interactions found were: In hydroaeroponics, CIAT899 was more efficient in symbiosis with Flamingo than with Coco, whereas the native *S. fredii* was more efficient with Coco than with Flamingo. The latter was more tolerant to salinity than Coco with CIAT899, but not with the native *S. fredii* 1a6, i.e. Flamingo could be selected for a higher SNF potential than the local cultivar, but it might not express this difference with native rhizobia. In contrast, BAT477 was superior to Coco under 25 mM NaCl with the three rhizobia (Jebara et al., 2001a).

In sand culture, the plant growth and the symbiotic efficiency were 5 to 8 times lower than in hydroaeroponics (Jebara et al., 2001a). Thus, sand culture may not be adequate to select the most efficient symbiotic partners. Moreover, the symbiosis ranking for SNF was different from the one found in hydroaeroponics, probably because of differences in the sensitivity to water deficiency, presumed to be a major limitation of the sand culture. In hydroaeroponics, specific interactions were also found among white-seeded beans: T815 and the local cultivar Linex harbored less than 20 nod pl^{-1} with the native *R.* sp. *Phaseolus* LR14 and more than 150 with CIAT899, but Diego harbored more than 200 nod pl^{-1} whatever the rhizobia (Rey-Poiroux et al. 2002).

In this project, no correlation was found between the variation of tolerance of native rhizobia for growth in free-living culture under salinity, and that for symbiotic efficiency under moderate salinity. This contrasts with the higher N_2 fixation levels observed by Ben Abdelkhalek et al. (2003) with the Tn5 mutants, which displayed higher tolerance to salinity than the parent *R. etli* CFN42.

NODULE METABOLISM AND O_2 PERMEABILITY

Since Salt-tolerant symbioses were selected partly in controlled environment, physiological assays were performed to search for mechanisms involved in the tolerance. Nodule physiology is characterized by an intense respiration of photosynthetic sucrose to support the reduction of N_2 by nitrogenase. A central role is played by phosphoenol pyruvate carboxylase (PEPC), which links bicarbonate with trioses into C4 organic acids for the energetic supply of bacteroids, the incorporation of fixed N into amino acids, and the regulations of osmotic pressure and pH. A low and variable permeability of the nodule cortex controls the entry of O_2 (Minchin 1997). This gas is toxic for nitrogenase though it is intensely demanded for its ATP-dependent N_2-reduction. Higher nodule PEPC and malate dehydrogenase activities were found in NaCl-tolerant symbioses (Pliego et al. 2003). Nodule permeability was increased under salinity (Jebara et al. 2001b). The latter contrasted with the NaCl-induced decrease in nodule permeability that was associated with a contraction of nodule inner-cortex cells in soybean, i.e. the 2-4 cell layers

between the vascular traces and the most internal non-infected cells surrounding the infected zone (Serraj et al. 1995).

A nodule-specific carbonic anhydrase (CA) catalysing the hydration of CO_2 into the substrate of PEPC was found to express in the nodule cortex of *Medicago sativa* (de la Pena et al. 1997). The corresponding gene in common bean, namely *Pvca*, was cloned from a mRNA extract of nodule-cortex and used as a probe with an *in situ* hybridization methodology to test whether or not CA plays a role in nodule adaptation to salinity. The CA expression was found precisely in nodule inner-cortex cells (Schump et al. 2003). It might be associated with higher nodule permeability due to P deficiency, although it varied among nodules and plants of a single experiment. These results were consistent with an osmoregulatory regulation of nodule permeability: the CA would be involved in the synthesis of malate subsequently accumulated in the vacuoles as an osmoticum. Malate would act also as a counter-anion of the putative K^+ accumulation driving the water accumulation and subsequent turgidity of the inner cortex cells (Drevon et al. 1995). Aquaporin was the other candidate gene chosen in this project since tonoplastic aquaporins were found by immunolocalization to be over-expressed in the nodule inner cortex (Serraj et al. 1998).

CONCLUSION AND FUTURE PROSPECTS

Figure 1 is an attempt to illustrate the experimental links between disciplines within FYSAME, which benefited from sharing common controls and

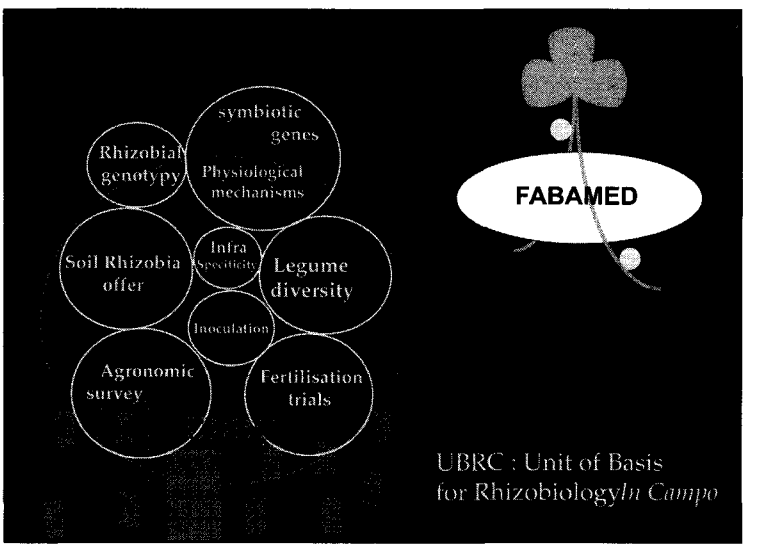

Figure 1. Interactions between disciplines in relation with field experiments in the FYSAME project.

promising results. In this project, the cooperation between bacteriologists and plant breeders was stimulated by describing the diversity of *Rhizobiaceae* species in production areas, and showing the specificity of their interaction with the selected bean lines. More interdisciplinary work is needed in this field. The screenings in controlled environment gave the opportunity for interaction with physiologists. It is now required to expand this link to functional genomics to identify candidate genes, and develop tools such as gene sequencing, in situ hybridization and immunolocalization. These will help to further elucidate the mechanisms involved in the improvement of the symbiorhizobial fixation of N_2 and tentatively extrapolate the findings to other grain legumes such as chickpea or fababean which are important sources of protein for humans in the Mediterranean basin.

Field level interaction with agronomists and producers will also require more attention to promote an integrated improvement of the symbiotic nitrogen fixation in grain legumes.

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III

Status of Legumes and SNF in South Asia and Africa

13

Exploring the Biological Potential of Indigenous African Legumes for Developing Agri-technologies and SMMEs to Alleviate Poverty in Rural Communities

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ABSTRACT

The flora of Africa is highly rich in species of the Leguminosae, which in many ways are the real riches of Africa. Increased legume research in Africa has the potential to reduce food shortages, produce market-oriented goods, and financially empower rural communities. Rooibos tea (*Aspalathus linearis*) and Honeybush tea (*Cyclopia* spp.) are examples of legume products that currently earn millions of dollars for the South African economy. Many of the *Vigna* species that are indigenous to Africa form high-protein seed and tubers for use as food, but have so far attracted very little research. Integrated studies of these under-promoted and under-utilized food and tea legumes have the potential to promote the development of agri-technologies that are urgently needed for the emergence of SMMEs, the key pillars for improving the quality of life in rural communities.

INTRODUCTION

Africa is home to about 43% of all the legume genera on this planet (Sprent 1998). Many of these are adapted to a wide range of environments (Sprent 2001), which include highly acidic nutrient-poor soils with very low rainfall (e.g. 50-75 mm per year in the Cedarberg region of South Africa). Irrespective of where they grow naturally, legumes have many uses as food, nutriceuticals, phytomedicines, pharmaceuticals, timber, fodder,

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bioremediators and environmental cleansers or detoxifiers. Consequently, the domestication and/or commercialization of native legumes could help improve the quality of life, alleviate poverty and economically empower rural communities.

The South African *fynbos*, a distinctive community of plants found within the Mediterranean type climate of the South Western Cape, is rich in flora, including legumes that are a source of herbal tea. *Aspalathus linearis* subsp. *linearis* (Rooibos tea) and *Cyclopia* spp. (Honeybush tea) are two N_2-fixing legumes that are currently developed for commercial tea production. The protein-rich seeds and tubers of other legumes of Southern Africa such as *Tylosema esculentum, Vigna lobatifolia, Vigna vexillata* and *Sphenostylis stenocarpa* (African yam bean) are used for food. Although the nutritional profiles of these legumes remain unknown due to lack of biochemical characterization, they are likely to be a great source of valuable nutriceuticals and essential amino acids. This paper examines the biological potential of indigenous African tea and food legumes as new market crops for poverty alleviation among rural communities through the establishment of business incubators.

ROOIBOS TEA (*ASPALATHUS LINEARIS* SUBSP. *LINEARIS*)

Biological Properties

Although seedlings of *A. linearis* subsp. *linearis* are sensitive to frost and snow, the mature plants are adapted to both cold winters and hot summers (Morton 1983), and their distribution confined to the Cedarberg mountains. The use of wild *A. linearis* plants as tea by the Khoi San was reported in 1772. The plant has since been domesticated and is presently cultivated as a commercial crop for export and local consumption (Morton 1983). Compared to oriental tea, Rooibos tea is caffeine-free and has significant medicinal value. It is often prescribed to alleviate nervous tension, allergies and various stomach and indigestion problems (Petereit et al. 1991). The tea is low in tannins and has been suggested to have anti-ageing effects due to its high concentration of anti-oxidants (Yoshikawa et al. 1990). Rooibos tea also contains various flavonoid molecules, including quercitrin and luteolin, which have antispasmodic properties (Snykers and Salemi 1974), aspalatin (a dihydroxychalcone), and the flavones orientin and isoorientin, which together account for its flavor and antioxidant effects (Robak and Gryglawski 1988). Rooibos tea is therefore a natural medicinal beverage that is rich in nutriceuticals, with great potential for establishing business incubators. Yet its increased production is constrained by many factors.

Production Potential and Constraints to Increased Yields of Rooibos Tea

The high concentration of flavonoids in Rooibos tea has caused it to become a favorite health supplement in Asia, Europe and North America, which together represent an expanding market for this legume. The total land cropped to *A. linearis* has increased dramatically with time (Fig. 1), which has in turn led to a marked increase in production levels (Fig. 2). Both cultivation and production have more than doubled since 1991 (Figs. 1 and 2).

The Rooibos tea plant nodulates freely with root-nodule bacteria, and its yield is largely dependent on the extent of this symbiosis. While *A. linearis* nodulates with bacterial isolates from other *Aspalathus* species such as *A. cordata, A. divaricata, A. biflora, A. hispida, A. retroflexa, A. abietina, A. flexuosa* and *A. ericifolia,* it does not do so with bacteria isolated from *A. forbesii* and *A. salteri;* nor does it nodulate with rhizobia from other tropical legumes (Dakora 1998). This tea legume also does not nodulate with bacteria in soil inocula collected from different locations in South Africa and Namibia (Dakora 1998),

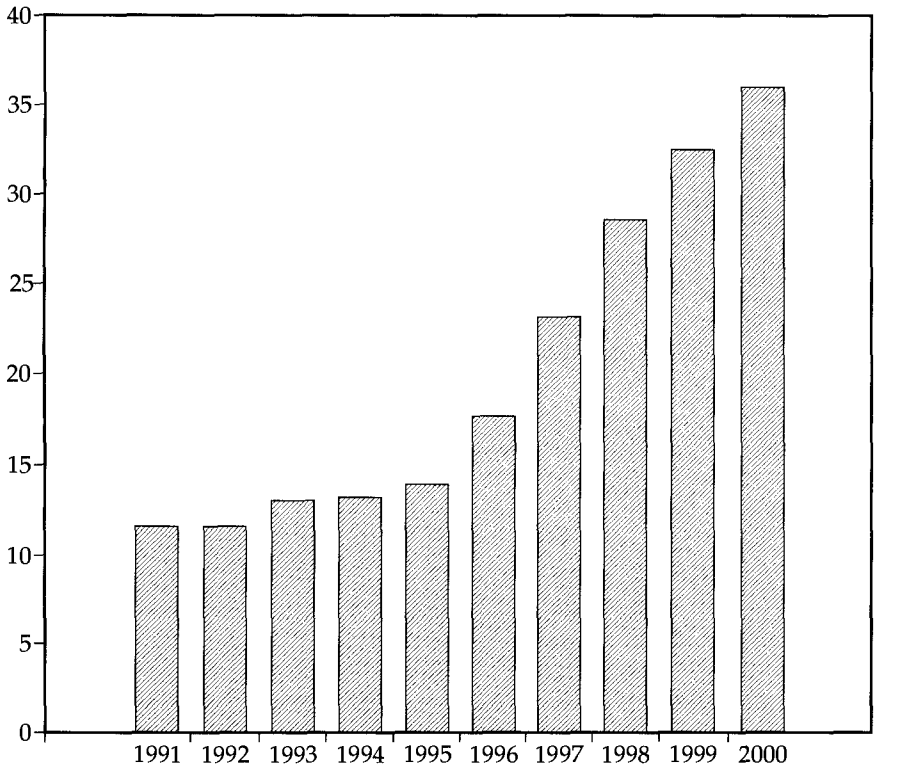

Figure 1. Land area under Rooibos tea cultivation.

indicating that bacterial inoculants must be produced and supplied to farmers if cultivation of this legume is to expand beyond its area of endemicity in the Western Cape of South Africa. Fortunately, laboratory studies have shown that N_2-fixing bacteria isolated from *A. linearis* can tolerate acidity as high as pH 3 and as low as pH 8 (Muofhe and Dakora 1998), indicating that *A. linearis*-nodulating bacteria can adapt to a wide range of soil acidity. Although this does not eliminate the requirement for inoculation if it is to be grown outside the area of *A. linearis* endemicity, these findings indicate a high chance of strain survival following inoculant application to field plants.

In addition to identifying the best bacterial strain for inoculant production, there is a need to increase our understanding of the nutritional aspects of this tea legume. The soils supporting field growth of *A. linearis* are not only acidic (pH 2.9-4.5, see Muofhe and Dakora 1998), but also extremely nutrient poor. Consequently, provision of N, Ca and P to field plants grown in these soils markedly increased their growth, N nutrition and yield. The exogenous supply of P increased N_2 fixation by 85%. Interestingly, even in

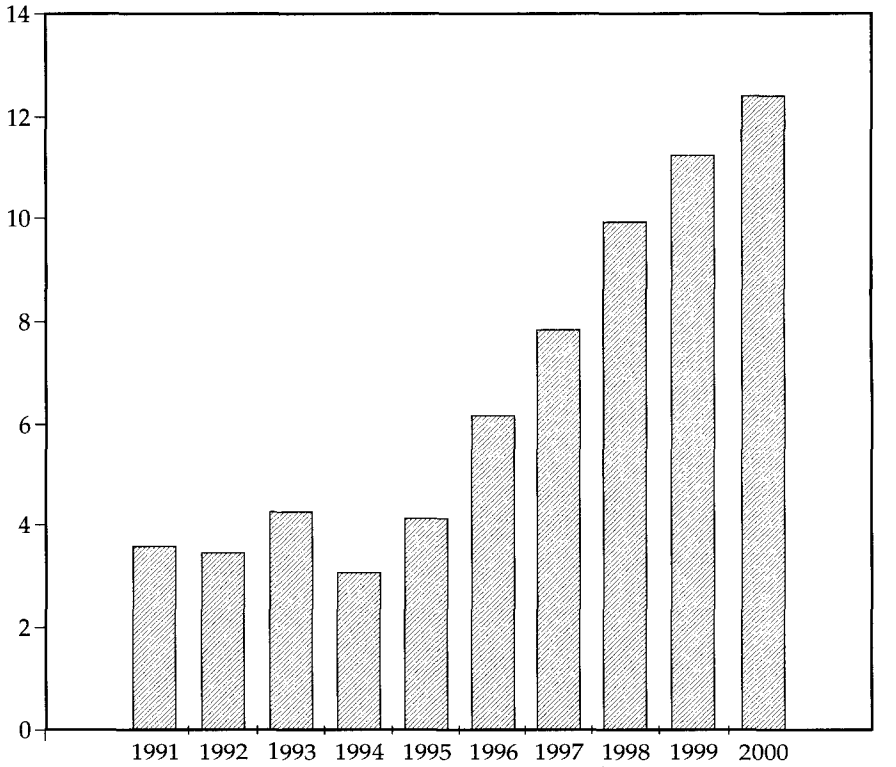

Figure 2. Annual Rooibos tea production.

unfertilized plots, the contribution of symbiotic N_2 fixation to the N economy of the ecosystem ranged from 105 kg N ha^{-1} for 1 yr-old plants to 128 kg N.ha^{-1} for 3 yr-old plants (Muofhe and Dakora 1999), clearly indicating the remarkable adaptation of this tea legume symbiosis to the very nutrient-poor, low pH conditions of the soil that it grows in.

HONEYBUSH TEA (*CYCLOPIA* SPP.)

Biogeography and Species Properties

The Honeybush tea (*Cyclopia* spp.) plant is another nodulating legume that is endemic to the Western Cape and is used for making tea. The genus *Cyclopia* consists of 14 species that grow in a variety of environments with differing soil ecologies throughout the Western Cape. The soils are characteristically sandy, nutrient-poor and highly acidic (pH 2-5) with total N often lower than 0.01%. As with *A. linearis*, the leaves and twigs of *Cyclopia* spp. have historically been used as a source of herbal tea by the local Khoi San people. Like Rooibos, Honeybush tea is also very rich in flavonoid compounds and is therefore used as a health supplement. Additionally, Rooibos and Honeybush tea are used in various culinary preparations and in the manufacture of baby foods. Consequently, the local and export markets of Honeybush tea have increased dramatically (Table 1), and this has necessitated a move from harvesting of wild *Cyclopia* for tea to its cultivation as a commercial crop. Today, the young Honeybush tea industry consists of 20 producers including 3 communities, 30 wild harvesters, 7 processors and 15 marketers.

Production Potential and Constraints to Increased Yields of Honeybush Tea

As shown in Table 1, the amount of Honeybush tea produced and sold has tripled since 1999, a clear indication of an expanding market for this tea. However, our lack of technical knowledge of the crop, including its production and sustainable harvesting methods, is a stumbling block given the high demand for the tea. For example, inadequate knowledge of the biology of this legume is a major limitation to increased tea yields. Being a perennial shrub, its cultivation requires seedling establishment in nurseries prior to field planting, and as a nodulating legume, *Cyclopia* seedlings must be inoculated with symbiotically efficient and competitive inoculant strains before planting in the field. These aspects have remained unresolved.

A recent study has shown that the soil bacteria that infect and nodulate *Cyclopia* are very closely related to members of *Mesorhizobium* and *Bradyrhizobium* (Spriggs and Dakora 2002). We also found that although Honeybush tea species exhibit nodulation specificity in terms of bacterial

Table 1. Honeybush tea sold per year since 1999 by the Honeybush tea growers association.

Year	Honeybush tea sale (t y^{-1})
1999	50
2000	100
2001	55
2002	152
2003	150 (estimated)

requirements, especially when dealing with isolates from grain legumes, they freely nodulate with bacterial strains isolated from other legume species that are similarly indigenous to the Western Cape. This means that expanding *Cyclopia* cultivation into new fields within the Western Cape will not require inoculation. However, expanding the industry into new areas outside the Western Cape will require the isolation, selection and production of high N_2-fixing inoculant strains for distribution to farmers.

Another problem limiting growth of the Honeybush tea industry is that so far no breeding program has been established to select for specific useful traits of *Cyclopia*. Thus, any screening of rhizobial strains for competitiveness and efficacy in N_2 fixation is currently done with uncharacterized wild accessions or ecotypes. For the industry to grow and expand into new non-endemic areas of South Africa and beyond would require selection of *Cyclopia* genotypes for useful traits, such as faster growth, nodulation promiscuity and/or tea quality, and then matching these genotypes with bacterial strains that have also been selected for superior symbiotic performance. Such an approach to host-strain selection, especially at the nursery level, would increase Honeybush tea production and benefit emerging small-scale farmers in the Western Cape. Not only is this method inexpensive and environmentally sustainable, it also fits into the low-input concept of those producing organically-grown Honeybush tea, an industry that is presently controlled by resource-poor, small-scale farmers.

Because it is organically grown, Honeybush tea has better taste and tea quality and it attracts a higher market price than conventionally produced tea. Thus, an increase in the production of organic Honeybush tea would mean higher incomes for families and households of small-scale farmers, a decrease in poverty among rural communities, and an improvement in their quality of life.

DEVELOPING AGRI-TECHNOLOGIES AND SMMES FOR ROOIBOS AND HONEYBUSH TEA IN RURAL SOUTH AFRICA

The current Rooibos and Honeybush tea industries consist of emerging small farmers and large commercial farmers. The former are resource-poor and produce their tea organically, while the latter use chemical inputs. The two

tea industries provide jobs for rural people at different stages of the enterprise, starting with land preparation, planting of seedlings, harvesting of leaves and twigs for tea, and during processing and packaging. On average, one major Rooibos tea farmer can employ up to 30 workers during the planting of seedlings, and another 30 during harvest. Small farmers, on the other hand, run household units. Thus, the more economically-empowered a tea farmer becomes in the expanding tea industry, the more jobs he potentially creates for rural communities.

The development of agri-technologies such as inoculant production, manufacturing of tea harvesters, and designing of equipment for tea processing, when combined with their sale and distribution to farmers, offers great opportunities for emergence of small, micro, and medium enterprises (SMMEs). As shown in Fig. 3, the chain of economic activities associated

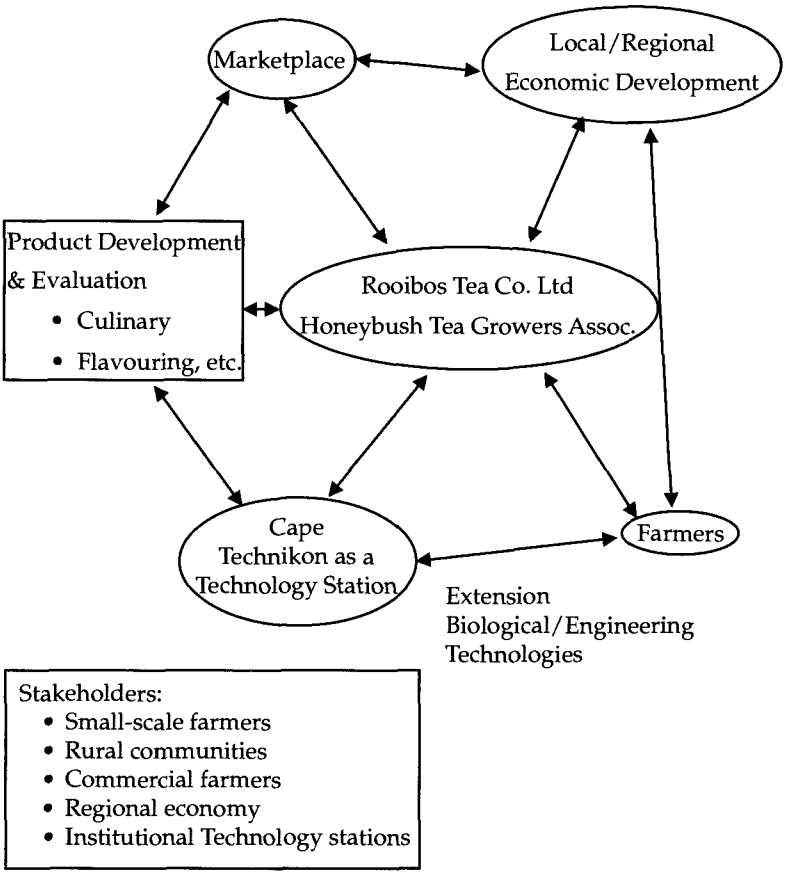

Figure 3. Interactive effects of developed agri-technologies for community empowerment via SMMEs.

with Rooibos tea or the Honeybush tea industry are vast, and can, at various stages, provide job opportunities for the rural unemployed, while creating wealth for entrepreneurs. So, from our knowledge of the growth and symbiotic performance of these tea legume species, to sustainable harvesting of leaves and tea processing, a number of SMMEs could emerge within rural communities where these tea species are cultivated. That, in turn, could lead to economic empowerment, improved quality of life, and ultimately poverty alleviation among the rural communities. In this way, our knowledge of N_2 fixation in these tea species would have transcended mere academic exploration into the realm of household economics, wealth creation, poverty alleviation and improvement in livelihoods. In our view, such an integrated approach stands a better chance of convincing donors to fund legume research than our current narrow focus on N_2 fixation per se.

DEVELOPING UNDER-UTILIZED INDIGENOUS AFRICAN FOOD LEGUMES AS NEW MARKET CROPS

In addition to the two tea species discussed here, there are many indigenous African legumes that are used for food, but have remained under-researched, under-promoted and under-utilized. Although they all serve as food security crops in sub-Saharan Africa, none is currently domesticated or cultivated. All these legumes are reported to grow in dry nutrient-poor soils, suggesting that their domestication and cultivation could expand agricultural activity into some of the presently unproductive marginal lands. Except for *V. vexillata* and *S. stenocarpa* which are nodulating (NAS 1979), and *T. esculentum* which is non-nodulating (Dakora et al. 1999), nothing is known about the symbiotic status of the remaining species (Table 2).

Interestingly, virtually all the legumes shown in Table 2 are creepers. So, they are not only effective cover crops for controlling soil erosion, they also serve as forage or fodder for game and livestock development. The nutritive value of these underutilized legumes is also reported to be very high (NAS 1979). For example, the grain of *T. esculentum* contains about 30-39% protein

Table 2. Some under-utilized African food legumes.

Legume	Geographic distribution	Edible parts
Vigna lobatifolia	Angola, Namibia, Botswana	tuber
Vigna fischeri	Malawi, Zambia, Kenya	tuber
Vigna reticulata	Malawi, Zambia	tuber
Vigna vexillata	Africa-wide	tuber
Sphenostylis stenocarpa	West, East, Central Africa	tuber, seed
Tylosema esculentum	Botswana, Namibia, South Africa	tuber, seed
Tylosema fassoglense	Botswana, Namibia, Angola, South Africa, Kenya	tuber, seed

Source: Dakora 1995.

relative to 38-40% in soybean, and 43% oil compared to 48% in groundnut. Being rich in linoleic acid, a nutritionally essential fatty acid, the dietary quality of seed oil from *T. esculentum* is also exceptionally high. This is in addition to the seed protein being quite rich in the essential amino acids lysine and methionine, about 5% and 0.7%, respectively.

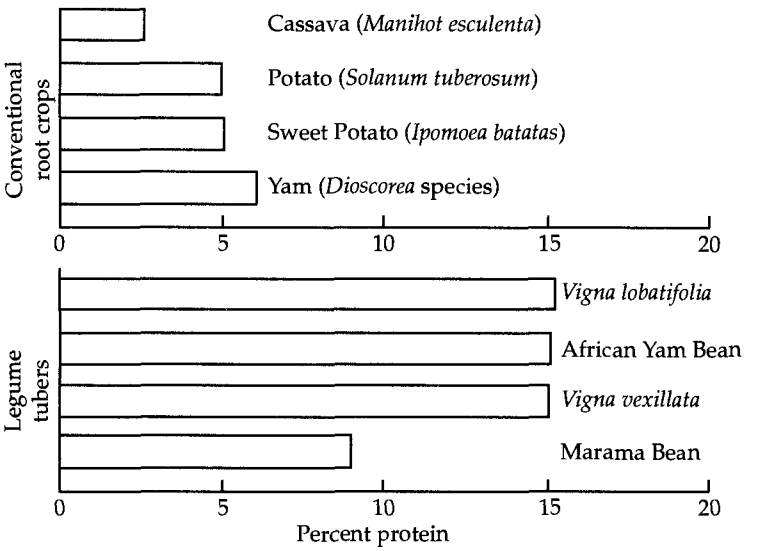

Figure 4. A comparison of the protein content of indigenous African legume tubers with conventional non-legume root tubers (NAS 1979).

As shown in Fig. 4, the edible tubers of these wild legumes are of higher nutritional value than most conventional tuber crops. The tubers of *V. lobatifolia*, *S. stenocarpa* and *V. vexillata*, for example, contain about 15% protein, a level six times that of cassava and three times that of the Irish potato or sweet potato (Fig. 4). Because very limited nutritional studies have been conducted on the tubers of underutilized African legumes, it is possible that their real value is yet to be discovered. *T. esculentum* is the main food legume of the Kalahari Bushman. Young tubers (1 to 3 yr-old) of this legume are either eaten raw, roasted or cooked. The taste is so delightfully unique that *T. esculentum* tubers may well be a major source of nutriceuticals. Domesticating, cultivating and characterizing the nutritional value of these legumes (Table 2) could lead to the development of new market crops.

CONCLUSION

In our view, the current approach to legume research is too narrowly based on symbiotic N_2 fixation. Measuring fixed N in legumes and estimating its

contribution to the N economy of soils are, on their own, insufficient to attract steady funding for legume research. To attract sustained funding for studies on legumes would require developing and adapting BNF technologies for commercialization through the formation of SMMEs in rural communities. Thus, an interdisciplinary approach to legume research is more likely to yield products that add value in the market place and with potential to improve the quality of life among rural communities in developing countries.

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Nitrogen Fixation in the Common Bean (*Phaseolus vulgaris*) – A Multilocational Inoculation Trial in Senegal

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ABSTRACT

A field experiment was carried out to investigate the response of the common bean (*Phaseolus vulgaris*) to inoculation with elite rhizobial strains in multilocational inoculation trials in Senegal. A positive response of common bean Nerina variety inoculated with *Rhizobium etli* ISRA 353 and *R. tropici* ISRA 554 was observed at all sites, average shoot dry weight and pod yield increases of +100% and +66% respectively were observed over the control plants. Significant differences have however been recorded between the two rhizobial inoculants in the shoot dry weight at three sites where ISRA 554 performed more (+ 42%) than ISRA 353 whereas differences in pod yields were observed only at one site where the highest value (+13%) was recorded with ISRA 554.

INTRODUCTION

The common bean (*Phaseolus vulgaris*) originated in the Americas (Gepts and Bliss 1988), and is an important legume crop worldwide. In Senegal, it is intensively cultivated during the cold dry period from October to March in a growing area called the Niayes zone, at sea level from 14°N to 16°N latitude. The cropping system used is an irrigated and intensive production of monocultured beans. In this system 300 kg of urea are applied per hectare to produce average pod yields of 4500 kg ha^{-1}. Diouf et al. (1999) have demonstrated the need for the common bean to be inoculated with rhizobial strains in the Niayes zone, which contains less than 10^3 g^{-1} soil of native

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rhizobia belonging to *Rhizobium etli* and *R. tropici* (Diouf et al. 2000). Two *Rhizobium* strains, ISRA 353 and ISRA 554,were selected – one from each of the species. The major objective of this study was to investigate the response of common bean to inoculation with these elite strains using multilocational inoculation trials in the Niayes zone.

MATERIALS AND METHODS

In 2000, a field experiment was carried out at four sites: Bambilor, Gorom I, km 50 and Sangalcam. The soil of these sites was sandy in texture and classified as Entisol (Soil Survey Staff 1987). Native rhizobia were counted by plant infection method (Brockwell et al. 1982; Vincent 1970) using the common bean as host. The seeds of the common bean variety Nerina and that of non-nodulating soybean (*Glycine max*) variety m129 (used as non-fixing control plant) were surface sterilized by immersion in 95% ethanol for 3 min and 0.1% $HgCl_2$ for 3 min and then washed with water. After washing, seeds were hand sown in a randomised completed block design with four replicates. The size of each plot was 1.35 x 2.55 m with 15 cm within and 45 cm between rows. Four treatments were given: (1) seeds of common bean inoculated with *Rhizobium etli* ISRA 353; (2) inoculated with *Rhizobium tropici* ISRA 554; (3) noninoculated and non-nitrogen treated seeds of common bean; and (4) noninoculated and nitrogen-treated seeds of common bean. Rhizobial inoculants were supplied as peat slurry containing 10^7 *Rhizobium* g^{-1} (0.2 g $seed^{-1}$ approx) and nitrogen fertilizer was applied as urea at 300 kg ha^{-1}. Within each subplot, a 0.60 x 0.45 m microplot was delimitated for the application of ^{15}N-labelled fertilizer solution, $(^{15}NH_4)_2SO_4$ containing 5 atom % ^{15}N excess. Unlabeled $(NH_4)_2SO_4$ was applied to the remaining plots. A basal fertilizer of 60 kg P ha^{-1} as triple superphosphate and 120 kg K ha^{-1} as KCl was added to all plots.

At maturity, all plants were harvested from the microplots. The harvested plants were separated into different parts. Shoot dry weight and pod yields were recorded. In addition, nitrogen (%N) and atom% ^{15}N excess (% ^{15}Nae) for the shoot were determined at the laboratory of soil biochemistry in ISRA-IRD centre of Bel Air, Dakar. Nitrogen fixation in the shoot (%Ndfa) was estimated using the isotope dilution equation (Fried and Middelboe 1977):

$$\%Ndfa = \left(1 - \frac{\%\ ^{15}Nae \text{ in common bean}}{\%\ ^{15}Nae \text{ in reference soybean}}\right) \times 100$$

Data were statistically analysed using the Newman and Keuls test.

RESULTS AND DISCUSSION

A positive response was observed at all sites – average shoot dry weight and pod yield increases of + 100% and + 66% respectively were observed over the control plants. The response supported the conclusions of Diouf et al. (1999), who reported the need for common bean to be inoculated with rhizobial strains in Niayes zone. The population of native rhizobia is low in these sites (Table 1) and the rhizobial inoculants were applied in quantities at least 1,000 times greater than the estimate number of native rhizobia. The response is similar to that of the field grown soybean (*Glycine max*) reported by Weaver and Frederick (1974) and in peanut (*Arachis hypogaea*) (Diatloff and Langford 1975).

Table 1. Soil characteristics of four sites of Niayes zone: Bambilor, Gorom I, km 50 and Sangalcam.

Characteristics	Bambilor	Gorom I	Km 50	Sangalcam
Sand (%)	91.2	96.5	70.0	87.0
Loam (%)	5.3	2.5	12.5	7.5
Clay (%)	3.5	1.0	17.3	5.5
pH (H_2O)	8.4	7.4	7.9	6.7
pH (KCl)	7.5	6.8	7.3	5.8
C(‰)	3.3	2.7	9.0	4.4
N(‰)	0.2	0.2	0.8	0.5
Available P (ppm)	73.0	150.0	278.0	82.0
No. of rhizobia g^{-1} soil.	10^2	10^2	10^2	10^3

Significant differences were recorded between the two rhizobial inoculants in the shoot dry weight at all sites, where ISRA 554 performed better (+42%) than ISRA 353. Differences in pod yields were observed only at km 50, where the highest value (+13%) was recorded with ISRA 554 (Table 2). The differential response between ISRA 353 and ISRA 554 rhizobial strains can be attributed to the differences in the effectiveness of the two *Rhizobium* species, *R. etli* and *R. tropici* (Diouf et al. 2000).

At all sites, N content and total N_2 were quite similar in N_2-treated and inoculated plants, and were higher than that in the control plants. Despite the presence of indigenous rhizobial strains, no nodulation was found on the harvested control plants in most of the selected sites, indicating that symbiotic nitrogen fixation did not occur in this treatment. In contrast, N_2 fixation occurred in the noninoculated plants receiving N fertilizer, showing thereby that a starter dose of N fertilizer is a prerequisite for N_2 fixation in these conditions. Diouf et al. (1999) have already proposed the application of 20 kg N ha^{-1} as a starter. In the present study, irrespective of the rhizobial strain used, the proportion and amount of fixed nitrogen were significantly higher in the inoculated plants than in the N_2-treated ones – on average, 70.9

Table 2. Dry weight, nitrogen yield, proportion (%Ndfa) and amount (Ndfa) of nitrogen derived from atmosphere of shoot and pod yield of field grown common bean (*Phaseolus vulgaris*) Nerina variety cultivated at four sites in Niayes zone and inoculated with *Rhizobium etli* ISRA 353 and *Rhizobium tropici* ISRA 554 or supplied with nitrogen fertilizer (300 kg urea ha^{-1}).

		Shoot										
Site	**Treatments**	**Dry weight**	**%N**	**Total N (kg ha^{-1})**	**%^{15}Nae**	**%Ndfa**	**%Ndff**	**%Ndfs**	**Ndfa (kg ha^{-1})**	**Ndff (kg ha^{-1})**	**Ndfs (kg ha^{-1})**	**Pod yield (kg ha^{-1})**
Bambilor	Control	700c	2.0b	17.0b	0.90a	–	–	–	–	–	–	3100b
	Nitrogen (300 kg urea ha^{-1})	1600ab	2.7ab	44.3a	0.65b	22.3b	25.6a	52.1a	10.3b	12a	23a	4700a
	R. etli ISRA 353	1300b	3.4a	46.4a	0.27c	67.5a	9.8b	22.7b	32.2a	5b	11b	4800a
	R. tropici ISRA 554	1800a	3.1a	54.7a	0.25c	70.3a	9.0b	20.7b	39.7a	5b	11b	5100a
Gorom I	Control	700b	2.1b	16.7b	0.87a	–	–	–	–	–	–	2200c
	Nitrogen (300 kg urea ha^{-1})	1200a	3.8a	48.9a	0.66b	21.6b	16.2a	62.2a	11.4b	8a	32a	3400b
	R. etli ISRA 353	1400a	3.2a	49.0a	0.19c	76.5a	11.3b	12.2b	37.1a	5a	6b	4600a
	R. tropici ISRA 554	1500a	3.0a	42.2a	0.25c	69.8a	10.5b	19.7b	30.3a	5a	8b	5000a
Km 50	Control	800b	2.1b	13.8b	0.81a	3.7c	15.6	80.7a	0.6c	2b	11b	3400c
	Nitrogen (300 kg urea ha^{-1})	900b	3.9a	40.2a	0.68b	19.7b	26.9a	53.4b	8.5b	12a	23a	4300b
	R. etli ISRA 353	1100b	3.6a	45.2a	0.22c	72.8a	10.8b	16.4c	33.1a	5b	8b	4500b
	R. tropici ISRA 554	1600a	3.2a	44.6a	0.22c	73.7a	10.8b	15.5c	33.4a	5b	7b	5100a
Sangalcam	Control	700c	2.0b	15.0b	0.92a	–	–	–	–	–	–	3.0b
	Nitrogen (300 kg urea ha^{-1})	1800a	2.4ab	40.2a	0.66b	21.4b	10.3a	68.3a	9.6b	4a	29a	4.4a
	R. etli ISRA 353	1200b	3.3a	45.3a	0.23c	71.7a	10.5a	17.8b	32.6a	5a	8b	4.5a
	R. tropici ISRA 554	1700a	3.1a	49.5a	0.29c	65.0a	9.8a	25.2b	32.4a	5a	12b	4.7a

In each column and for each site, values followed by the same letter do not differ significantly at $p = 0.05$

vs 21.2 %Ndfa and 33.9 vs 10.0 kgN ha^{-1}. This indicated the beneficial effect of the rhizobial inoculants, which resulted in significantly less soil nitrogen uptake in the inoculated plants (18.8 vs 65.8 %Ndfs), thereby sparing soil nitrogen.

The response of common bean to rhizobial inoculation has been frequently studied. Poor nodulation, low efficiency of rhizobial strains and lack of response to field inoculation have been frequently reported (Graham 1981, Salez and Saint Macary 1987, Hungria et al. 1999). However, some promising results have also been reported. Twelve international bean inoculation trials sown in seven countries in Latin America from 1978 to 1979 showed significant yield responses with 39-61% increase above controls without nitrogen (Graham et al. 1981). Maingi et al. (2001) reported that inoculation of common bean with a commercial rhizobial inoculum was effective and improved yields in semi-arid southeast Kenya

This multilocational common bean inoculation trial was initiated in Senegal in 2000. The results from this study demonstrated a response of common bean to inoculation with rhizobial strains and opened prospects for using rhizobial inoculants on a large scale in the Niayes zone to improve N_2 fixation.

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Design, Development and Promotion of Soybean BNF Technology in Zimbabwe: Closing the Loop

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ABSTRACT

Biological nitrogen fixation contributes significant amounts of N into both managed and natural ecosystems and forms the basis for the age-old practice of rotating legumes with other crops. Benefits of legume N_2 fixation include protein nutrition, soil fertility improvement and savings on fertilizer costs as well as cash income from sale of crop surpluses. The packaging and use of superior N_2-fixing rhizobial strains as commercial legume inoculants is a relatively cheap cost-effective technology widely adopted by large-scale but not smallholder farmers in Zimbabwe. We report on a promotion program that used soybean as a vehicle to convey the multiple benefits of BNF technologies to poor smallholder farmers through a multi-faceted research-extension-promotion effort. The primary objective was to strengthen rural food security of smallholder farmers through exploitation of soybean BNF for soil fertility improvement against rising input costs. The main elements of the promotion strategy included training farmers and extension staff in technology application, and demonstration of the tangible multiple benefits and facilitation of inputs/outputs marketing, all backed by a parallel program of adaptive research. The basic promotion concept used was that of creating a closed loop with four links: training (in BNF technology application), production (of soybean), processing and marketing (TPPM). Coordination of stakeholder activities was and continues to be a critical component of the promotion effort. A conceptual framework linking various elements (BNF technology, food security, soil fertility, cash income) was used to guide and focus both the promotion and research components. The rate of adoption of soybean BNF among smallholders has been near exponential (from 50 in 1996 to >10,000 in 2000). This paper outlines the mechanisms used in the promotion of soybean technologies, the responses of smallholder farmers and the prospects for wider scaling up.

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INTRODUCTION

Nitrogen deficiency is the main limiting factor for high cereal yields in sub-Saharan Africa and yet the majority of smallholder farmers use very little N fertilizer. Biological nitrogen fixation (BNF) contributes significant quantities of nitrogen (N) to both natural and managed ecosystems and offers a relatively cheap alternative source of N for resource-poor farmers. Exploitation of BNF technologies in farming systems of Africa requires identification of appropriate N_2-fixing legumes that have multiple benefits to ensure adoption by risk-averse rural communities. There is need to develop a research agenda that identifies appropriate BNF technologies (e.g. effective legume-*Rhizobium* combinations) that can be readily adopted by farmers with immediate demonstrable benefits to ensure adoption. Such research efforts will need to be linked to appropriate extension programs that ensure that target communities benefit in tangible ways

Traditional legumes such as groundnuts (*Arachis hypogaea*), cowpea (*Vigna unguiculata*) and Bambara nuts (*Vigna subterraneae*) that rely on BNF and contribute residual fertility to soils are low-yielding and are often viewed as minor crops. Yields of these legumes have failed to respond consistently to inoculation with commercial rhizobial strains. Soybean, a relatively new legume in Africa, responds well to rhizobial inoculation and fixes large amounts of N even in fairly marginal soils (Kasasa 1999, Musiyiwa 2001). The multiple benefits of soybean include soil fertility improvement, protein nutrition for humans and livestock, and cash income from sales of grain and processed products. Soybean is now grown in several parts of sub-Saharan Africa including Malawi, Nigeria, Zambia and Zimbabwe, where it is making significant contributions to rural livelihoods. Due to limited inoculant production capacity in most African countries, promiscuous soybean varieties that effectively nodulate with indigenous rhizobia have been successfully grown without inoculants, demonstrating their potential as vehicles for conveying the benefits of BNF to poor and marginalized communities (Mpepereki et al. 2000).

HISTORICAL PERSPECTIVE ON SOYBEAN IN ZIMBABWE

Soybean was introduced into Zimbabwe (then Southern Rhodesia) in the 1930s as a green manure crop and later for forage. Large-scale commercial production started in the 1960s when a breeding program and a *Rhizobium* inoculant factory were also established (Corby 1967). The crop was not promoted among smallholder black farmers, most of whom had been relocated on to marginal, often sandy soils in low rainfall areas unsuitable for soybean production. Apart from the real agroecological limitations, soybean production, with its requirement for rhizobial inoculants that need

refrigeration, was considered too sophisticated for the African peasant farmers who had no knowledge on how to process it for food.

After political independence in 1980, the government adopted a policy of encouraging smallholder farmers to increase crop production through various inputs and marketing support programs. By the 1990s, smallholder farmers were contributing over 70% of the national maize and cotton production. A soybean BNF promotion program targeted at Hurungwe West district in northern Zimbabwe in the late 1980s boosted farmer interest, production and consumption of soybean, which all declined when project support ended in 1989 (Whingwiri 1996, Mudimu 1998). Smallholder farm communities however continued to face limited dietary protein sources, general declining soil fertility and poor household incomes, against a background of increasing mineral N fertilizer prices following World Bank/IMF-induced removal of government subsidies. A two-day stakeholders' workshop that was held at the University of Zimbabwe in February 1996 to examine the potential for promiscuous soybean for smallholder farmers recommended two major activities. First it resolved that research be initiated to characterize indigenous soybean rhizobia, the potential for promiscuous soybean and to quantify amounts of N_2 fixed and the residual fertility benefits for maize grown in rotation. Second, it resolved to extend soybean technologies (use of rhizobial inoculants and promiscuous varieties for BNF, production, processing, utilization and later inputs/outputs marketing) to smallholder farmers. A National Soybean Promotion Task Force was set up with representation from farmers' organizations, private industry, NGOs and public institutions (research, extension, university). The Task Force was to be convened by AGRITEX with overall coordination by the University of Zimbabwe Faculty of Agriculture. The Task Force objectives included promotion of appropriate research, training farmers in production and processing of soybean, and coordinating the activities of stakeholders.

This paper outlines the mechanisms used to promote soybean technologies, the scale of operations, feedback from farmers, constraints and opportunities and the potential for scaling up.

CONCEPTUAL FRAMEWORK

The context was that of smallholder cropping systems characterized by low productivity due to low soil fertility with N as a major limiting nutrient. Biological N fixation was identified as a potential tool to address N deficiency in these systems. Soybean was chosen as the candidate legume because of its high N_2-fixing potential and soil improving properties, food value as a protein and vegetable oil source, relatively low production costs and high market

value. The place of soybean BNF in the total food production system of a typical smallholder farm was identified. This was an essential step to ensure that the technology would address real food security concerns of farmers, a critical element for successful adoption. The conceptual framework illustrated below (Fig. 1) shows the main linkage loops and benefits from soybean BNF in an integrated maize-based crop-livestock system.

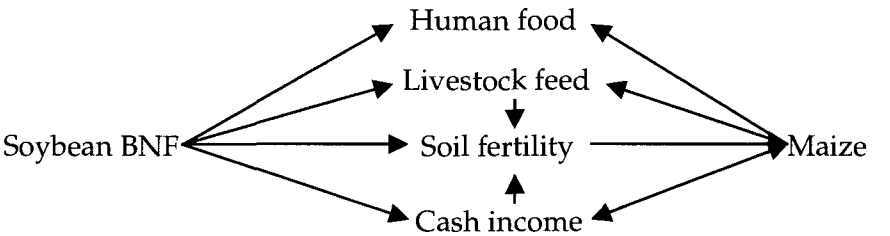

Figure 1. Soybean BNF links to food security in a maize-based farming system (adapted from Mpepereki et al. 2000).

TRANSLATING THE CONCEPT INTO AN OPERATIONAL MODEL

Methods

Graduate students were engaged to conduct research to quantify N inputs from promiscuous and commercially inoculated soybean into the cropping system and to measure and demonstrate the residual soil fertility benefits for maize in subsequent seasons. Research was conducted to establish the prevalence and symbiotic effectiveness of indigenous rhizobia on both promiscuous and specific soybean varieties and the adaptability of the latter to the more agroecologically marginal smallholder areas. Experiments were conducted on-station under researcher- and on-farm under farmer-extension management. This meant that researcher-managed, detailed, replicated field experiments were placed on a few farms selected for their representative soil types, while a larger number of simple plus/minus treatment trials were run under farmer management with extension officers monitoring them. Rhizobial inoculation, liming and basal compound fertilizers, and promiscuous versus specific nodulating soybean varieties were tested. Both farmers and extension personnel helped to set up and monitor experiments and gained valuable experience and confidence in managing a soybean crop. Scientific data obtained was used to strengthen the extension messages that had hitherto been extrapolated from work done in large-scale commercial production under somewhat different agroclimatic conditions.

For the promotion aspect, the main strategies were: **training** of farmers and extension staff on how to apply rhizobial inoculants and how to grow, weed and harvest soybean; **facilitating access to inputs**; setting up **demonstrations** that **involved farmers and extension staff**; regular **follow-ups**; and **communication in local language** at all times. Train-the-trainer workshops targeted extension staff in AGRITEX, NGO personnel and farmer leaders identified by their organizations and employed a hands-on practical approach. Topics included how to store and apply rhizobial inoculants, use of promiscuous varieties where inoculants are unavailable, how to check if nodules are effective, and identification of pests and diseases and their control.

Training was consolidated by a vigorous program of field discussion days that acted as a field laboratory course for farmers and extension staff. Local traditional and political leaders were invited to field days to raise the profile of the promotion and facilitate more rapid evaluation and information dissemination on soybean BNF technologies.

Access to inputs was facilitated by mobilizing stakeholders in agro-industries to deliberately stock inputs in rural areas where demand had been created by the training and promotion program. Introductory input packages containing seed, *Rhizobium* inoculants, lime and basal fertilizers enough for 0.1 ha per soybean variety were put together and distributed at a slightly subsidized cost to ensure that the crop had all required nutrients.

SOYBEAN AS HUMAN FOOD

Soybean has up to 40% protein, 20% oil, 30% carbohydrate, 10% fiber and numerous vitamins and antioxidants, making it perhaps the single most nutritionally balanced food crop available today in terms of both energy and protein. Combined with maize, soybean provides a complete diet including all essential amino acids. The potential of soybean to reduce or altogether eliminate the incidence of malnutrition is very significant and makes it an attractive legume to introduce across many African environments with compatible agroecological conditions. The Soybean Promotion Task Force in Zimbabwe set up a team of food scientists and extension specialists who first identified and then developed simple ways to eliminate anti-nutrition factors found in soybean. They developed various recipes and ran numerous soybean processing training workshops at rural service centers and train-the-trainer workshops for rural women. Various women's groups subsequently adapted and developed their own new recipes compatible with local food tastes and preferences. The most significant development is that rural communities now use soybean to substitute for many expensive grocery items that include soy milk, soy 'coffee', soy flour for making cakes, bread, pastries and nutritious soy-based relishes replacing expensive meat. Currently research is continuing both at UZ and at the Department of Research and Extension (Ministry of Agriculture, Zimbabwe) to look at the

quality, nutritional value and shelf life of some of the soy-based foods being prepared by village women with a view to scaling up and commercializing production. The Nigerian experience in the above regard has some important lessons for Zimbabwe (N Sanginga, pers. comm.). The overall outcome of training women in processing and utilization of soybean as food at household level has seen a huge increase in the number of families adopting the crop.

SOYBEAN AS LIVESTOCK FEED

Farmers growing soybeans generate large amounts of crop residues, which contain more protein than e.g. maize stover. Many are already feeding the residues and grain to livestock. The benefits to the farmers include better draught power for timely land preparations at the start of the cropping season, and higher milk, meat and hide production. The manure from animals is an important soil organic matter amendment for resource-poor farmers who cannot afford adequate mineral fertilizers. For many African farmers livestock represent a critical investment, 'money in the bank', as they can be sold to meet food and other needs of the family.

Marketing and Incomes

The lowest loop on our conceptual model (Fig. 1.) emphasises the link between soybean BNF and cash income. Each soybean harvest provides food, seed and surplus for sale. In the Zimbabwean model, the Task Force working with farmers' organizations, commodity brokers and processors put in place marketing arrangements to ensure that farmers receive fair prices for their soybean grain. The key to success has been effective load consolidation, identification of lucrative markets and affordable transport. Initially volumes were small and marketing costs very high but as more farmers took up the crop, volumes increased allowing economies of scale to come into play. A comprehensive study to analyze the economic potential of soybean showed that there are '...potential benefits ... for smallholder farmers, particularly the poorer smallholders ...' in Zimbabwe (Rusike et al. 2000).

Coordination

For the adoption rate to be sustained there was need to coordinate the efforts of the many stakeholders that are involved. Figure 2 illustrates the range of possible linkages that are involved in the soybean BNF research-extension program in Zimbabwe. To facilitate coordination a unit was established in 2000. Its major function was to provide technical backup and training to various groups engaged in soybean production and to mobilize stakeholders. Currently stakeholders are setting up a soybean development trust takeover

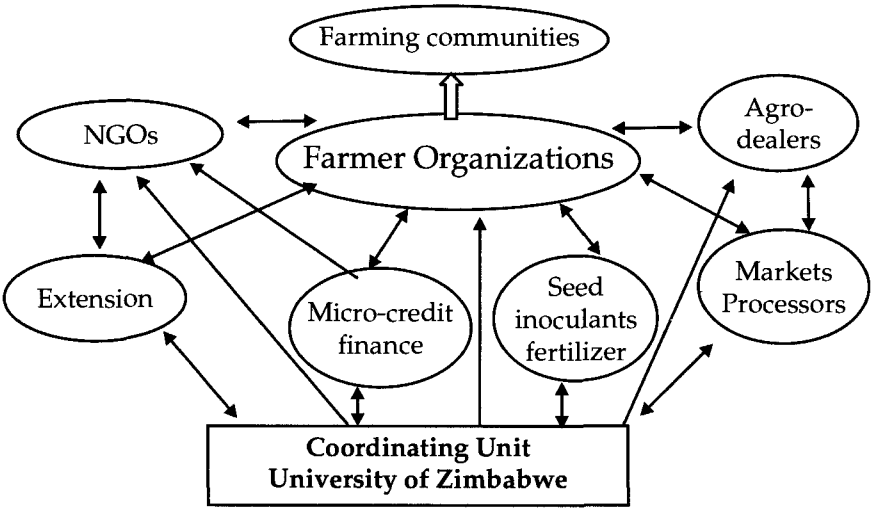

Figure 2. Coordination linkages of stakeholders in the soybean promotion program in Zimbabwe. Key activities in the links include training, information exchange, adaptive research and movement of inputs, outputs and cash.

coordination of all stakeholder activities in research, production, processing, marketing and training in the entire country.

OUTCOMES

In general the research-extension program has successfully introduced and brought benefits of soybean BNF to thousands of smallholder families in Zimbabwe. Promiscuous soybean has enabled farmers with no access to commercial inoculants also to adopt soybean. Up to 50% of soybean produced in Hurungwe district in northern Zimbabwe in the last four seasons (1998-2001) was promiscuous, while in Zambia and Malawi promiscuous Magoye still forms the backbone of smallholder soybean production (Javaheri 1996). Promiscuous soybean forms the bulk of varieties planted in Nigeria. We summarize below results from various research initiatives undertaken within the conceptual frameworks described to illustrate the kinds of information being generated. Readers can refer to the original publications for greater detail.

One of the studies quantified amounts of N_2 fixed by different soybean varieties under field conditions (Table 1) as the initial stage in demonstrating the residual soil fertility benefits of rotating maize with soybean.

Yields of maize after soybean were significantly higher than maize after maize, demonstrating significant residual fertility effects of soybean (Table 2). This is a positive contribution to sustainable food production and security, as maize is the staple for many sub-Saharan communities. These residual

Table 1. Nitrogen yields from promiscuous and specific (improved) soybean varieties at Hotera smallholder farm, Hurungwe, Zimbabwe (1997).

Soybean Variety	% N derived from fixation		Fixed N (kg ha^{-1})	
	–Inoculation	+ Inoculation	–Inoculation	+ Inoculation
Magoye	91	90	73	58
Local	90	90	57	58
Roan	91	88	63	66
Nyala	92	82	46	58
s.e.d	3.8		15.8	

'Magoye' and 'Local' are promiscuous; 'Roan' and 'Nyala' are specific commercial varieties; s.e.d. Standard error.
(Adapted from Kasasa et al. 1998).

Table 2. Maize yields for two seasons following soybean in a sandy loam soil in a smallholder farm, Hurungwe, Zimbabwe (1998/99).

Soybean variety (96/97)	Soybean biomass incorporated (t ha-1)	Maize yields (t ha-1)	
		97/98	98/99
Magoye (prom.)	5.4	2.3	1.2
Local (prom.)	4.9	2.1	1.4
Roan (spec.)	3.2	1.8	0.9
Nyala (spec.)	2.8	1.4	0.8
Maize control	Nil	0.2	0.2

fertility effects on maize have been consistently obtained under farmer management and boosted adoption of soybean BNF against a background of rising mineral N fertilizer prices and depreciating local currencies. Mineral fertilizer inputs (e.g. Ca, Mg, P, K, M) will continue to be required to prevent nutrient mining of soils.

An important benefit of soybean BNF has been the boost in household incomes from grain sales by farmers (Table 3). A critical element in the promotion program has been the consolidation of loads so that the economies of scale have enabled the relatively small production of each farmer to be sold on the lucrative commodity exchange as part of a large parcel. Thus the conceptual model for promoting BNF includes produce marketing as a key element.

A study of the economic potential of soybean showed that the crop was most profitable for the poorest farmers as it had lower input costs but gave the highest return on investment (Rusike et al. 2000). Poor farmers who adopted soybean for the first time between 1997 and 2001 have testified that they earned more money from soybean sales than from any other crop that they have ever grown (Table 4). The significant boost in family dietary protein availability (Table 4) is a critical element of household food security, a key benefit of BNF among rural communities where poor nutrition among the HIV-infected is contributing to the high death toll from AIDS-related illnesses.

Table 3. Soybean grain sales by smallholder farmers from four locations over 4 marketing seasons in Zimbabwe.

Location	Amounts sold (metric tons)			
	96/97	97/98	98/99	99/2000
Guruve	6.2	53	153	210
Kazangarare	58.0	280	475	580
Sadza	0.5	3.5	7	10
Senge	0.2	6	11	18
Total sold	64.9	342.5	646	818

Note: Only sales facilitated by the Soybean Promotion Task Force are reflected; farmers also used other marketing outlets.

Table 4. Grain, protein and cash returns from soybean for Tapera smallholder farm in Zimbabwe (1998).

Soybean variety	Total grain yield (kg ha^{-1})	Protein from 15% seed retained (kg ha^{-1})	Cash grain sale (US$ equivalent)
Magoye	2100	126	471
Local	1900	114	302
Roan	2800	168	496
Nyala	3100	186	560

Average smallholder planting: 0.4 ha; average yield: 0.8 t ha-1; average price: US$360/ton (2001).

CONCLUSIONS

Our experiences developing and implementing a research-extension model for promoting BNF technology among peasant farmers in Zimbabwe offer lessons for similar initiatives in developing countries. Previous experiences of promoting promiscuous soybean in Nigeria (N Sanginga, pers. comm.), Malawi and Zambia (Mpepereki et al. 2000) also point to the need for integrated approaches that address both the scientific-technological and socioeconomic aspects in a holistic way (closing the loop). Demonstration of multiple benefits of N_2-fixing soybeans, use of promiscuous varieties, training women in home processing, adapting soybean to local diets, and facilitating inputs/outputs marketing, all carried out in the context of a clear conceptual framework with stakeholder participation, have resulted in rapid adoption of soybean by thousands of smallholder farmers, thereby strengthening their food security in a sustainable way. An integrated program of adaptive and applied research to support the soybean BNF promotion initiative has provided a scientific basis for a technical backup service to adopting farmers. The success of such a promotion program depends on the number of actual and demonstrable benefits to the smallholders and the commitment of all stakeholders to implement its various facets in a coordinated way. Marketing both in terms of inputs and outputs is a key driving force for soybean BNF

technology adoption. More BNF grant funds must go into activities that directly benefit farm families than project personnel salaries and per diems. Legume BNF *can* make a significant positive difference to rural livelihoods.

ACKNOWLEDGEMENTS

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Current Status of Food Legume Production and Use of Biological Nitrogen Fixation in Ethiopia

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ABSTRACT

Ethiopia is one of the largest food legume producing countries of the world and ranks first in Africa. The total food legume production in Ethiopia has increased significantly since the 1998/1999-crop season. The area under legumes increased from 0.87 million ha in 1998/99 to 1.23 million ha in 2000/2001 while production increased from 7.32 to 10.7 million quintals. This increase in area and production has come because of increased prices, attractive export markets and an increase in fertilizer price. Improved varieties are in production and newly released ones are being popularized. Biological nitrogen fixation (BNF) studies are underway for major food legume crops such as faba bean, chickpea, field pea, lentils and haricot bean. The National Soils Laboratory and Holeta Research Centers have made several collections of *Rhizobium* strains. Currently, over 247 strains are in our National Soils Laboratory. Among these, those screened and identified for their efficiency are being promoted through demonstration and popularization. Both the Ethiopian Agricultural Research Organization (EARO) and Institute of Biodiversity Conservation and Research (IBCR) have developed strategies to collect, conserve and evaluate the strains.

INTRODUCTION

Ethiopia is among the largest food legume producing countries of the world and ranks first in Africa in both area and production. The total food legume production in Ethiopia has been increasing since the 1998/1999 crop season (Table 1). This increase in area and production has come about as a result of high demand in both domestic and export markets. The National Pulse Improvement Program of Ethiopia has put in an effort to improve productivity of pulses in collaboration with international research institutes, mainly ICARDA, ICRISAT, CIAT and IITA. Tremendous progress has been made in

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Table 1. Areas (ha) of the major highland pulses in Ethiopia.

Area (ha)	1992	1995	1997	1998	1999	2000	2001	2002
Faba bean	238	343	337	329	293	359	426	369
Field pea	107	163	204	158	142	152	205	175
Chickpea	110	179	150	150	168	184	212	185
Lentil	39	62	83	53	48	72	90	60
Grasspea	65	85	76	104	–	–	–	85
Total	559	832	850	794	651	767	933	874

Source: Central Statistical Abstract of 2001 and 2002.

developing and releasing varieties with their agronomic packages for different agroecological zones of the country. To alleviate the major production constraints, resistant/tolerant varieties were developed and released, helping farmers to better manage the major diseases in food legumes.

Biological nitrogen fixation is very essential and has played a pivotal role in maintaining soil fertility for centuries. In recognition of this important contribution, BNF has been one of the major components of the Ethiopian research programs, although there has been a change in focus of late. Research on BNF in Ethiopia was started in the early 1980s with major emphasis on collection of nodules, isolation, and testing of strains of *Rhizobium* from collected nodules (Beyene 1988).

PROGRESS IN RESEARCH ON BNF

In the early 1980s, the Department of Soil Science of the Institute of Agriculture Research (IAR) established a Soil Microbiology Unit at its Holetta Agricultural Research Center, which was responsible for coordinating all the research on BNF in Ethiopia. The Unit developed some laboratory facilities and also linked itself with the International Atomic Energy Agency (IAEA) in Austria, International Center for Agricultural Research in the Dry Areas (ICARDA) in Syria, and Nitrogen Fixation by Tropical Agricultural Legumes (NifTAL) in the USA for possible financial and technical backstopping. According to Beyene (1988), 110 isolates were from *P. sativum*, *V. faba*, and *L. culinaris*; while 20 isolates were from *P. vulgaris*; 328 isolates from cowpea, soybean and chickpea and 34 isolates from *Trifolium spp* were collected by the Nazareth (Melkassa) Research Center. After senior microbiologists left the Melkassa and Holeta Research Centers, research in microbiology almost ended, but the laboratory technicians at these centers continued to maintain the collections.

Subsequently, the IAR became the Ethiopian Agricultural Research Organization (EARO), to coordinate all the National Research System in the country. EARO brought the National Soils Laboratory, Forestry Research, Animal Health Research and Fishery Research Centers from the Ministry of Agriculture (MoA) and the Debre Zeit Agricultural Research Center from the Alemaya University under its purview.

Currently, senior researchers at Holeta and National Soils Laboratory of EARO are fully involved in the collection, isolation, testing and promoting strains that were found effective at fixing N_2 and increasing yield. An attempt is being made to strengthen the microbiology work in Ethiopia. Many research facilities have been improved and biotechnology work is also linked to this laboratory. To date, the nationally coordinated BNF program has identified effective *Rhizobium* strains in chickpea and faba beans while efforts are being made to do the same for other food legumes such as *P. vulgaris*, cowpea, grass pea, field pea and fenugreek. Currently, Holeta, Adet, Sheno, Sirinka and Debre Zeit Agricultural Research Centers are involved in BNF research as cooperators while the National Soils laboratory of EARO located in Addis Ababa coordinates the overall activities. This laboratory also provides training for extension workers and development agents that are involved at different stages of BNF promotion.

COMMERCIALIZATION OF BNF

The EARO gives much priority to promoting the use of biofertilizers (*Rhizobium* inoculants) as a low-cost technology for small-scale subsistence farmers to supplement plant nutrients for crop plants. The successful laboratory-scale production of inoculants in the National Soils Laboratory, Microbiology Unit has shown promise. Selection of carriers is going on and the future activities will be to upscale their production. Intensive training is given to the extension and development agents (DAs) to promote and commercialize the products.

FUTURE RESEARCH STRATEGY IN BNF

Generally, most soils in Ethiopia contain rhizobia capable of producing nodules in the major food legume crops that have been grown in the country for centuries, except for those that are highly specific in their rhizobial requirements and have not been previously grown in a given region. Beck et al. (1993) and Stanforth et al. (1994) indicated that nodulation and effective N_2 fixation are determined by several factors such as environment (soil moisture and others), competition among strains, and interactions between genotypes and *Rhizobium* strains. Therefore, the future strategy will be to:

- strengthen collection, characterization, evaluation, documentation and conservation, which will be the joint efforts of EARO and IBCR.
- screen under different stresses: dry, water-logging, acidic and saline soils to identify effective strains that would match with stress-tolerant genotypes of crops

- screen *Rhizobium* strains for persistence to perform uniformly from season to season.
- study the presence or absence of competitors or antagonistic effects in different agroecological zones and soils.
- develop effective and less costly carriers from local materials

It is known that genetic diversity exists in both crop germplasm accessions and rhizobial strains/collections in their gene pools. Therefore, their interactions are very useful during selection of crop genotypes and rhizobial strains. This has been already observed in chickpea trials carried out at Debre Zeit, Akaki and other testing sites in Ethiopia. Crops like chickpea, lentil and grasspea are usually grown on residual soil moisture and are exposed to terminal drought. Hence, selection will focus on identifying genotypes and *Rhizobium* strains that tolerate terminal drought and are efficient in fixing N_2 during flowering and pod development stages to support the plants

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Status, Constraints and Perspectives of Biological Nitrogen Fixation Technology in Egypt

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ABSTRACT

Microbial processes are essential to sustainable crop production systems. Adoption of BNF can ensure greater environmental biosafety, improvement of crop production and low inputs of expensive hazard chemicals. Biofertilization is well established in developed countries, while few farmers in developing countries use it, although it helps reduce inputs of chemical fertilizers. In Egypt, large amounts of N-fertilizers find their way to drainage water and eventually to drinking water, and are thus hazardous to health and the environment. Application of BNF systems started 45 years ago by inoculation of legume seeds with rhizobia, of rice fields with cyanobacteria and more recently, Azospirilla for various crops. Their utilization is mostly based on high efficiency in BNF and PGP activities. Egyptian research institutes are currently working on advanced biotechnology and genetic engineering programs that include activities related to BNF. This paper discusses constraints facing the application of BNF technology and offers suggestions for enhancing it. These include: (1) developing rapid assays that can predict performance of formulations, (2) evaluating compounds that promote cell survival on inoculated seed, (3) determining effects of amendments, chemical fertilizers and pesticides, (4) developing new extension strategies and initiating innovate informative methods statistically sound in design, (5) adoption of molecular biological methods to identify desirable characteristics, manipulate and introduce more effective and efficient strains for specific inoculation purposes, (6) exploring new associations, and (7) strengthening communication between researchers and extension experts.

INTRODUCTION

Microbial processes are essential to sustainable crop production systems. Much can be done by optimization of microbial processes for nutrient dynamics, pathogenesis and even at a system level without knowing the

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microbes involved. Soil-plant management techniques can shift the processes to conditions more favorable for agricultural sustainability and low input productivity. To achieve these specific goals in utilizing BNF systems, the following are necessary: (1) a search for safe biofertilizers to improve crop productivity and soil fertility, thereby reducing the need for excessive inputs of expensive and hazardous agrochemicals; (2) identification of methods to discover novel metabolic traits, and understand and harness N_2 fixers' diversity using natural and human managed systems; (3) extensive studies on the factors affecting BNF, host-plant selection for high BNF and rhizobial ecology; and (4) discussion of the opportunities for collaborative research on production, testing, application and monitoring of marketing constraints facing BNF technology.

CURRENT STATUS OF BNF UTILIZATION TECHNOLOGY IN EGYPT

While BNF inoculant-manufacturing industries and marketing of inoculants are well established in Europe, North America, and Australia, only some farmers in developing countries practice biofertilization of their fields. In Egypt, about 6 million acres (~60% of the available agricultural land use area) are cultivated annually with summer and winter legumes including faba bean, lentil, lupine, chickpea, bean, alfalfa, soybean and clover. Considering that the N-demand for these crops ranges between 175 and 300 kg N ha^{-1}, the amounts of fertilizer N added to the soil to meet this demand range from 420 to 720 thousand tons per year, equivalent to 1.98-3.39 million tons ammonium sulfate, 2.46-4.22 of calcium nitrate or 1.20-2.06 million tons of ammonium nitrate (Yanni 1999, unpublished data). Unfortunately, large amounts of these chemicals find their way to groundwater, drainage water runoff, and eventually drinking water. Attempts were begun about 40 years ago to reduce application of nitrogen fertilizers by performing large-scale seed or soil inoculation with rhizobia (Abdel-Aziz 2001, Yanni 1990 and 1998). Several field application trials demonstrated that biofertilization can increase crop production while decreasing the inputs of chemical fertilizers, thereby reducing the demands on high energy-consuming chemical industries, and helping restore ecosystem health (Yanni and Abd El-Fattah 1999). Although legume inoculation with rhizobia and rice inoculation with cyanobacteria and azospirilla, for instance, are an accepted and mature agricultural practice, utilization and transfer of the technology is still going slowly due to reasons that will be discussed later. Table 1 illustrates the progress in production of the major legume crops in Egypt throughout the last decade, and will give an idea of the successful development of BNF technology. The increase in productivity of unit area could be related to application of integrated crop management including: (1) new high-yielding and pest-resistant varieties; (2) better fertilization practices including starter

Table 1. Progress in production of the major legume crops in Egypt, 1992-2001.

	Seed yield (t ha-1)			Cultivated area (ha)		
Crop	1992-1996	1997-2001	Increase (%)	1998	2001	Increase (%)
Faba bean	2.31	2.80	20.9	134139	139708	4.15
Lentil	1.44	1.51	4.7	2252	2003	- 11.1
Chickpea	1.64	1.82	10.9	8440	7121	- 15.6
Soybean	2.67	2.81	5.3	—	—	—
Peanut	10.78	12.28	13.9	43582	63347	45.4

chemical nitrogen and nodulation with indigenous or inoculated symbiotic N_2 fixers, phosphatic fertilizers and/or inoculation with phosphate-dissolving bacteria; (3) foliar or soil application of trace elements and pesticides including herbicides, insecticides and fungicides; and (4) water management including surface, sprinkle and foliar irrigation with modified drainage system.

The research and application program included studies on various cultivars of legume fodder crops including four new varieties of alfalfa, four of Egyptian clover, one of guar and one of fodder lupine. These new varieties are now being tested for responses to inoculation with different strains of N_2-fixing bacteria. In addition, new soybean varieties that are resistant to the cotton leaf worm have been widely released to minimize use of insecticides and thus reduce agricultural input costs. Decayed organic matter from residues of legume, cereal and other field crops is being used, to reduce application of chemical fertilizers, maintain soil fertility, control environmental pollution and thus move towards agricultural sustainability. The program included establishment of a powerful research program for postharvest management to reduce loss of storage yield, quality control, and detection of contamination with aflatoxins that are hazardous to man and animals. Special emphasis was directed to production of new fodders from combinations of different agricultural wastes and recycling of other crop residues (mainly rice straw) in production of on-farm organic manure using advanced microbial preparations. An advanced research program was begun for production of microbial preparations including symbiotic N_2 fixers for legumes and Azospirillum for cereal crops, and to assess the beneficial endophytic association between rhizobia and cereals. This research/technological program includes quality control during different production stages, effects of storage, transportation and proper methods of field application. However, until now, only less than 5% of the area of legumes annually receives biofertilizer preparations, creating high potential for an active BNF program that would help establish new production strategies for major field crops.

International collaboration on BNF in Egypt started at the beginning of the 1980s. A strong collaboration was established with the NifTal project,

targeting a stronger BNF research/technology program. This involved inoculation of different legume crops with locally isolated cultures of symbiotic N_2 fixers, a field-testing program to compare effects of inoculation with indigenous vs imported rhizobial strains, and field management that supported maximum benefit from field inoculation. Besides, there was a strong research and application program with the Indian Agricultural Research Institute (IARI), New Delhi, which resulted in the setting up of mass production of cyanobacterial inocula for rice fields using the open-air soil culture method. The contribution of the late Prof. GS Venkataraman in establishment of this program cannot be forgotten. Other activities included enhancement of production of major cereal and legume crops through multidisciplinary research and application and bringing together experts of different areas in agricultural research with extension specialists. This resulted in the development of inocula now available in the market: rhizobia for legumes, cyanobacteria for rice and Azospirilla for legumes and cereals. Most of the research done has shown a positive response of crops to inoculation using the proper strain(s) (Yanni 1990, 1992 and 1998), and confirmed the importance of nodulated legumes in maintaining soil fertility through agronomic rotations involving both cereal and legume crops. Thus, the potential benefit of using efficient BNF inocula is high, considering that at present the area sown annually with inoculated legume or cereal seeds, or inoculated through soil application of inocula (like in the case of inoculation of rice fields with cyanobacteria), does not exceed 5% of the total cultivated area. Ninety percent of the total area can thus be considered the target area for efficient extension programs on biofertilization with BNF inocula. Economists have estimated that this practice can save the country about US$ 112 million per year in terms of savings on N-fertilizers (around 810,000 t yr^{-1}) (Abdel-Aziz 2001), despite indirect benefits due to decreasing environmental pollution, enhanced human and animal health and increasing work abilities.

Although N cycling in Egyptian rice farming systems has received considerable attention over the years, an active research program has now been established that addresses emerging issues such as stimulation of rice growth by *Rhizobium leguminosarum* bv. trifolii (Yanni et al. 1997, 2001; Dazzo et al. 2000 and Fig.1). Future research areas that can be focused upon are the interactions between application of BNF biofertilizers and the dynamics of soil structural changes; water content and diffusional constraints of nutrients compartmentalization in inoculated plants comparing with their noninoculated counterparts; and coupling of N cycling processes and linkage of microbial community structure to these.

In the last two decades, Egypt initiated programs to establish research institutions working in the areas of advanced biotechnology and genetic engineering, including activities related to BNF (Moawad 2001). Another

major collaborative effort was launched in 1996 and sponsored by the US-Egypt Science and Technology Joint Fund in which major priority areas were identified and more than forty-four collaborative research projects including BNF research and technology transfer were set up. It is clear then that BNF is still considered a promising research area in the focus of agricultural sustainability for enhancement of crop production without increasing chemical fertilizer inputs and environmental pollution. (Moawad 2001, Tiedje 2001).

BIOSAFETY OF BNF BIOFERTILIZERS

Some of the microorganisms used or planned to be used as biofertilizers in different farming systems, including those in Egypt, may adversely affect humans, animals, plants and/or the environment. For instance, although most strains of *Rhizobium leguminosarum* bv. trifolii isolated from within surface sterilized rice roots were effective N_2-fixing symbionts of clover, inoculation with some of them was proved to adversely affect the growth of

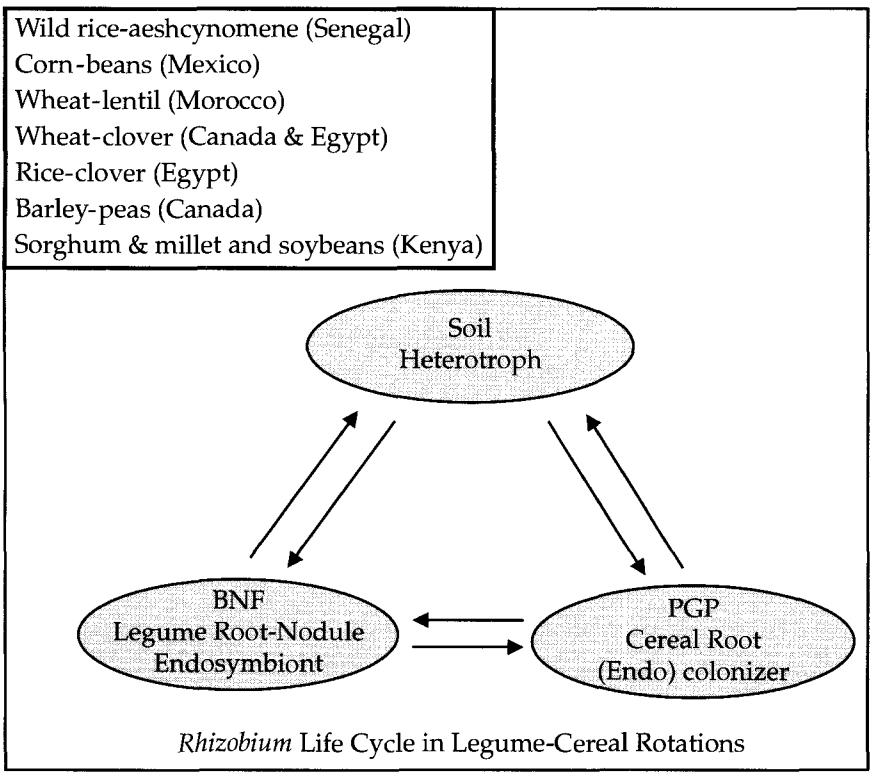


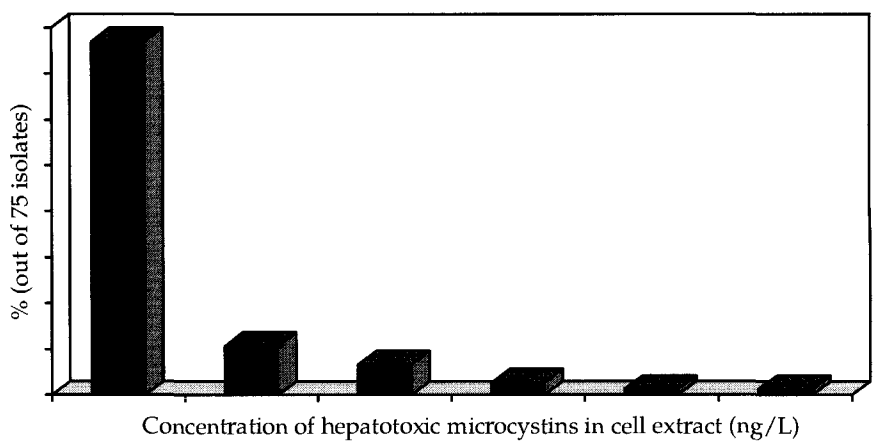
Figure 1. PGP *Rhizobium*-cereal associations.

rice traditionally following clover in the same crop rotation. On the contrary, some of these rice varieties root-adapted rhizobia indigenous to the same soil in the Nile delta were active endophytic plant-growth promoting rhizobacteria (PGPR) within rice roots but $Nod^+ Fix^-$ (borderline pathogens) on clover (Yanni et al 1997 and Yanni and Dazzo 2001). Some of the strains were found to be both Nod^+Fix^+ on clover and PGPR on rice (Table 2). Thus, utilization of inocula as biofertilizers requires their careful evaluation, including studies to assess their effects on other legume and cereal crops

Table 2. Examples of undesirable characteristics of certain rice endophytic strains of *Rhizobium leguminosurum* bv.trifolii (Rlt) isolated in the Nile delta recognized through PGP bioassays under gnotobiotic conditions.*

Rlt strain	Response on clover	Response on rice
E 3	Nod^+ Fix^+ (Good clover growth)	PGP^- (Inhibitor)
E 13	Nod $^+Fix^-$ (Pathogenic-Lethal)	PGP^+ (Good rice growth)

*Recognition of the adverse effects of the inoculant strain on non-target plant host avoids potentially catastrophic consequences in future field application. Both of the two strains cannot be used for biofertilization of clover or rice in a rotation containing the two crops.
Source: Yanni and Dazzo 2001.



About 77% of the isolates (of 75 isolates used in the study, axis Y) registered up to 500 ng/L (A) of their cell extracts. About 23% of them (17 out of 75) were found capable of producing more than 500 ng/L having varied toxin-producing abilities. 10.7% scored concentrations from 500 to 1000 ng/L (B), and 6.7% 1000 to 1500 ng/L (C). Extracts of two isolates contained up to 2000 ng/L (D), one registered 2800 ng/L (E) and another scored 4240 ng/L (F).

Source: Yanni and Carmichael 1998.

Figure 2. Screening of cyanobacteria belonging to *Anabaena* and *Nostoc* isolated from rice fields and water resources of the Nile delta for production of hepatotoxic microsystins.

sharing the same crop rotation, to avoid potentially catastrophic consequences in field application. In addition, some isolates of N_2-fixing cyanobacteria belonging to the genera *Nostoc* and *Anabeana* which are currently used as rice biofertilizer, were found capable of producing hepatotoxic microcystins and neurotoxins in lab cultures and natural habitats. In one study (Yanni and Carmichael 1998), 23 out of 75 cyanobacterial isolates collected from soil, rice fields and water resources in different localities in the Nile delta were found to be active hepatotoxin producers, and thus hazardous if included in a biofertilizer preparation (Fig. 2). Another serious issue is that some microbial preparations introduced recently for utilization in some farming systems contain unknown microorganisms. Considering that some of them may be human pathogens, toxins producers, plant growth inhibitors, or poorly tested genetically engineered microorganisms, utilizing these preparations could be hazardous and their introduction to the environment must not be taken lightly (Yanni and Dazzo 2001). Future multinational research studies and collaborative activities would do well to gain a broader-based understanding of the ecology of candidate biofertilizer strains and ensure their biosafety before release into the agroecosystem.

REASONS FOR LOW ADOPTION OF THE BNF BIOFERTILIZATION TECHNOLOGY

Reasons for the current low adoption rates of the BNF biofertilization technology in most developing countries include:

1. REASONS FOR WHICH SOLUTIONS CAN BE FOUND THROUGH IMPROVEMENTS IN TECHNOLOGY

- Agriculture uses few – if any – modern production inputs.
- Deficiency in the extension and technology transfer programs.
- Poor performance of some biofertilizer preparations. In one study, half of the inocula from 12 countries had less than 10^8 viable cells g^{-1} representing a population density where yield response is compromised (Bottomley and Myrold 2001). The farmer then no longer trusts the technology for sound and low cost fertilization management.
- Lack of advanced facilities for fast and effective quality control during and after manufacture, and for monitoring the candidate dynamics after field application.
- Inaccurate seed inoculation procedures; faulty preparing of the proper soil preparation; lack of appropriate water content in seed bed; and incorrect times, rates and methods of application of chemical fertilizers and pesticides needed to support successful biofertilization.

- Lack of information about the potential of indigenous N-fixers, their biodiversity, infectivity, N_2-fixing efficiencies and competitiveness to inoculated strains.
- Most of the agronomic studies to evaluate the efficacy of field inoculation lie in a "black box" category of research, recording positive results from indirect evidence. The infectiveness, effectiveness, and competitiveness of the inoculum strains against their indigenous counterparts thus need to be explored more fully.

2. REASONS THAT REQUIRE POLICY INTERVENTIONS

- There is no fixed data describing the magnitude of adverse effects related to excessive use of chemical fertilizers on man, animal, plant and environment and the overall effects on national economy.
- Extension programs for biofertilization are mostly taken lightly, especially where poorly-educated farmers can access large amounts of locally produced or imported chemical fertilizers in the market. The farmers prefer chemical N-fertilizers as they induce faster changes in plant features and growth responses, in despite of their potentially adverse effects.
- Neither the less-educated farmer nor agronomists and policy makers care enough about environmental pollution caused by excessive use of chemical fertilizers. This needs some governmental regulations and interventions.

HIGH PRIORITY TOPICS AND FUTURE AREAS OF MULTINATIONAL COLLABORATION

Attempts are being continued for developing and evaluating different inocula for distribution among farmers. These include developing rapid laboratory and greenhouse assays for quality; biosafety; predicting performance of formulations and evaluating compounds that promote cell survival in preparations; inoculated seed and those present in soil; besides determining effects of amendments, chemical fertilizers, and pesticides on growth of BNF biofertilizers candidates. Our high priority areas and future multinational collaboration should focus also on how to:

- Put the BNF technology in a proper scientific framework containing qualified research and extension personnel. Until now, there is a relatively wide gap between research work and extension services on BNF.
- Adopt methods that enable monitoring the process on field scale using scientific procedures that are agronomically and statistically sound in design.

- Reduce crop production costs for the low-income farmer and consumer.
- Relieve the environmental stress due to excessive use of chemical fertilizers, targeting a clean environment.
- Produce efficient inoculants that are capable of surviving and competing with native populations under the different extremes of temperature, dryness, salinity, and even excessive application of fertilizers and pesticides.
- Adopt molecular biological methods to identify desirable characteristics, and manipulate and introduce more effective and efficient strains for specific inoculation purposes in cropping systems based on legume-cereal rotations.
- Assess *Rhizobium* biodiversity for developing long-term solutions to interstrain competition and poor nodulation of certain legumes (e.g., beans).
- Screen biodiversity of PGP- rhizobacteria indigenous to soils for beneficial traits under field conditions, explore new associations and capitalize on their diversity, assess underlying mechanisms of growth-promotion, and investigate their ecology (survival, colonization, endophytic state, biogeography, and dispersal).
- Investigate the benefits vs. tradeoffs of mixed consortia inoculants.
- Investigate fundamental management central to utilization of BNF biofertilizers, including input management, crop management and evaluation methods.
- Monitor economical, environment-friendly and hygienic benefits of partial or complete substitution of chemical fertilizers by BNF biofertilizers.
- Strengthen the communications between BNF experts concerning active, effective and modern research and extension programs.

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Institutional Learning: From BNF Technologies to BNF Innovation Systems

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ABSTRACT

This paper presents the changes essential in our understanding of innovation, the organization and processes of innovation, and the assessment of their impact, which are crucial to the success of BNF. One of the significant reasons for the limited success of BNF in the past can be traced to the inadequate understanding of agricultural innovation systems, and the selective and linear perception of the complex processes of innovation. This paper argues that the capacity of the innovation system to respond to complex technological contexts will now determine the success of BNF. The current rekindling of interest in BNF is a process internal to agricultural innovation systems. This paper presents an 'innovation systems' analytical framework that addresses institutional and technological changes necessary for the generation and utilization of BNF. The systems perspective is essential because BNF innovation systems straddle both biological and social systems. It demands fundamental changes in the linear model of R&D that compartmentalizes innovation into research, extension and adoption boxes/organizations. An iterative institutional and technological learning process, building partnerships with relevant actors (not limited to participation in adoption) is necessary. The paper analyses the division between technological and social realms in conventional BNF technology generation. It reveals that a BNF innovation system makes demands from all stakeholders – professionals and society in general – to enable the nesting of BNF into larger system dynamics and land management goals. Research managers and policy makers must now identify and deliberate a range of incentives, capabilities and facilitation processes or institutional reforms that will bring the diverse actors together and enable their learning and evolution as part of the BNF innovation system.

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INTRODUCTION

This paper argues that institutional learning is essential for participants in the biological nitrogen fixation (BNF) innovation system. Successful BNF innovations demand a major shift in our research and development paradigm, from conventional linear technology generation and diffusion to nonlinear innovation systems. At its simplest, the concept of an innovation system states that innovations emerge from evolving systems of participants involved in research and the application of research findings. The shift to innovation systems is often perceived as building bridges or linkages or partnerships. We argue here that it is more than mere redesigning linkages or building bridges. Understanding and participating effectively in a nonlinear innovation system demands that we analyze the material that bridges are made of.

In other words, the nonlinear innovation systems in the real world demand changes within science as well as the linkages between science and its social contexts. It demands (among other things) a major capacity-building exercise within the scientific research components of innovation systems. The BNF community has to make an attempt to understand and change the way BNF technology policies are made, research conducted, development and distribution organized, local technological contexts studied, farmers/actors engaged and enthused into utilizing the technology, and so on as is specific for each context. This need not and cannot be accomplished by the scientific community alone. An innovation system approach brings other actors and partners with different skills and experiences; they can help the research community and the evolution of the BNF innovation process.

A striking feature of the current interest in BNF is that it has been generated from thought processes and requirements within organized agricultural research. This time round the pressure for BNF is not fuel price hikes, unreliability of political petrochemical relationships, or even the guilt-ridden attention we paid to the non-cereal i.e., leguminous crops and cropping systems that sustain the rural poor. What makes the BNF initiative different now (compared to its rather disappointing institutional history) is its emphasis of the following: sustainable soil fertility, adaptation to environmental concerns, participatory methods for better adoption, socioeconomic limitations of soil fertility management, and policy options for narrowing the soil fertility gap (ICRISAT 2002).

From the Romans, through Hellriegel and Wilfarth, to Beijerinck and several organizations, experts and networks, BNF has come a long way. One of the reasons for the limited success of BNF in the past can be traced to our flawed understanding of agricultural innovation systems, our selective perception of and exclusively 'economic' assessment of the complex process of innovation. BNF poses a major challenge to agricultural innovation as we

know and practice it in our IARCs and NARSs. This paper argues that the capacity of the innovation systems to respond to the complex technological contexts of BNF (host-specificities, local soil management practices, soil-crop relationships, and sociocultural and political constraints in BNF technology generation and utilization) will now determine the success of BNF.

> (S)uccessful innovation, as a rule, is based on diversity, on opportunity grasping, and especially on mobilizing creativity among people who are willing to run with a brilliant idea, even if it is still flawed and underdeveloped. ... Different times and places call for new theories of innovation.
>
> Roling 2002 (foreword to Douthwaite 2002)

Why haven't millions of farmers across the world taken to BNF technologies? This paper attempts to answer this and some related questions. The lessons from these answers will then lead us to some crucial processes of thinking and learning that will help mobilize and sustain BNF innovation systems. To this end, it becomes necessary to distinguish between conventional BNF technologies and the BNF innovation systems that combine institutions and technologies. We start with an exploration of contemporary debates about innovation, which stress a systems perspective. This explanation of an innovation system is essential because this technology straddles the interface between biological and social systems. Understanding and targeting this technology for agricultural development or poverty alleviation requires this type of systemic engagement.

THEORIES OF INNOVATION

Linear and Systems Models of Innovation

At the heart of the challenges that the CGIAR is facing with respect to BNF is the implied need to embed technology and capacity development in a much broader set of relationships and contexts. We therefore locate this analysis within an innovation systems perspective. Both this perspective and the recent evolution of the science culture, characterized by partnerships, reveal the need to redraw conventional approaches to technology generation, development and policy.

The need to revise conventional approaches stems from an almost universal questioning of the conventional/linear model of knowledge production and utilization, where the traditional view is still to think in terms of a division of labor between 'knowledge search' and 'knowledge use.' The emergent view is that it no longer holds true that knowledge can be independently produced in specialized research organizations and that this

can then be transferred to the passive end users. A useful way of thinking about it is to focus on innovation (rather than research) in the broad sense of new knowledge of socioeconomic significance, and the systems of actors and institutions that give rise to such innovations. Innovation, as distinct from research and invention, is a much more complex process, often requiring technical, social and institutional changes involving actors across the conventional knowledge producer-user divide.

Innovation Systems Thinking: Origins, Principles and Contexts

The origin of innovation systems thinking can be traced to the idea of a 'national system of innovation' proposed by Freeman (1987) and Lundvall (1992). The innovation systems concept is now widely used in the policy process in developed countries, but has only recently started to be employed in relation to research policy in the developing countries (see for example, Hall et al. 2001).

Another way of making a similar point is proposed by Gibbons et al. (1994) in their much-cited discussion of mode-I and mode-II production of knowledge. In mode-I, knowledge is generated, often with government assistance, by a research community accountable to its disciplinary peers. The Gibbons thesis is that institutional changes in western societies (particularly where the market has started to eclipse the state as the primary decisionmaker) have forced science to become more socially embedded and less hierarchical, thus defining the mode-II type. The important point is that as societies and economic systems become ever more complex, the mode-I type of production of knowledge would become less able to respond to rapidly changing user contexts. Only by assuming the features of mode-II production of knowledge can systems be designed to cope with complexity and rapid change.

Lundvall (1992) identifies learning and the role of institutions as the critical components of innovation systems. He considers learning to be an interactive and thus socially embedded process, which cannot be understood without reference to its institutional and cultural context, usually in a national setting. The innovation system concept provides a framework for: (1) exploring patterns of partnerships; (2) revealing and managing the institutional context that governs these relationships and processes; (3) understanding research and innovation as a social process of learning; and (4) thinking about capacity development in a systems sense. On this last point, Velho (2002) observes that national systems of innovation, made up of actors which are not particularly strong, but where links between them are well developed, may operate more effectively than another system in which actors are strong but links between them are weak.

Table 1 presents the way innovation systems thinking has emerged and the way innovation policy with its focuses on the systems and processes of change has overshadowed earlier science and technology policy preoccupations of resource allocation. The shift stems to a large degree from the inadequacy of neoclassical economics traditions to deal with the evolutionary nature of technical change (Nelson and Winter 1983).

Increasingly, emphasis is being placed not only on knitting together different elements of national innovation systems, but also on embedding the planning of such endeavors in a wider constituency than only key scientific stakeholders. An innovation systems perspective brings together thinking from a broad set of theoretical canons that view development and change in systems terms. At its heart lies the contention that change – or innovation – results from and is shaped by the system of actors and institutional contexts in particular locations and particular points in time. A related recognition is that knowledge production and use is a highly context-specific affair. This has many analytical implications: the need to consider a range of activities and organizations related to research, particularly technology users, and how these might function collectively. There is also the need to locate research planning and implementation in the context of the norms, culture and political economy in which it takes place – i.e., the wider institutional context (Soderbaum 2000). Similarly, it is no longer useful to think of institutional and organizational arrangements for research as fixed or optimal – clearly these must evolve to suit local circumstances (ibid.). The evaluation of innovation performance also becomes much more context-specific relating the perspective of stakeholders and current imperatives, rather than either scientific peer review or economic justification alone.

INSTITUTIONS IN BNF INNOVATION SYSTEMS

We need a Veblenian perspective of institutions as rules or norms, to help us understand how the BNF technologies are located and behave within the innovation system. An understanding of this nature is necessary to explore how BNF, slated to be a pro-poor and successful technology, has thrived in research labs, research publications and conferences. But the purposeful exploitation of this technology in farmers' fields and pastures remains an unfulfilled target for agricultural science. We argue that a successful BNF innovation system demands institutional change.

'Institutions are sets of common habits, routines, established practices, rules, or laws that regulate the relations and interactions between individuals and groups.'(Edquist and Johnson 1997, p. 46) For instance, one of the characteristic agricultural research institutions is 'hierarchy'; say, of the natural sciences over the social sciences, of agricultural research over agricultural extension, of commodity production over natural resource

Table 1. Innovation systems under changing paradigms.

Period/ Paradigm	Conception of science	Who produces scientific knowledge	Model of technological change	Policy framework and policy tools	Tools for policy analysis and research evaluation	Model of North-South co-operation
Postwar period until beginning of 1960s "Science as an Engine for Progress"	Historically and socially neutral, follows its own internal logic	The scientists ("Republic of Science")	Linear relationship: basic research, applied research, technological development, innovation diffusion, economic progress and social welfare ("science push")	Focus on Science Policy: large-scale science funding; allocation of resources through institutional normative mechanisms, scientific merit	Peer review (sooner or later the "good" science finds out its practical application). Input indicators.	Problem-solving phase: find quick solutions to development problems through the use of human and financial resources of the Northern countries
1960s and 1970s "Science as Solution for Problems" (and also as "cause of problems")	Disputes about the neutrality of science	The scientists (but they must be directed and put in contact with the "demand")	Linear relationship: (the same as above, but "demand pull")	Science Policy and Technology Policy: Emphasis in resource allocation in terms of priorities (often by sector of activity) Science had to find a way to be used by technological development	Peer review plus output indicators (basically biblio-metric studies); role of S&T in economic growth; history of technology innovation at firm level (learning in TT)	Developing indigenous capacities of individuals (problem-solving and research capacities) in the recipient countries
1980s and 1990s "Science as a	Science wars (dispute between realism and relativism/cons	Scientists directly influenced by a complex	Complex – includes several actors, a diversity of institutions and	Emphasis on resources administration and allocation	Intensification of the peer review process, program assessment (concern with the	Generate new collaborative partnerships that benefit both sides

Contd.

Table 1. Contd.

Period/ Paradigm	Conception of science	Who produces scientific knowledge	Model of technological change	Policy framework and policy tools	Tools for policy analysis and research evaluation	Model of North-South co-operation
Source of Strategic Opportunity"	tructivism)	network of actors and its interests	processes (Technological trajectory subjected to "lock-in"– somewhat deterministic)	to strategic programs, interdisciplinary and collaborative research (national, institutional and disciplinary level) "alliances"	"impacts"), perspective and foresight	From supply-driven to demand-oriented (involvement of stakeholders by using participatory methods)
21st century "Science for the Benefit of Society" (back to the Baconian ideal)	Socially and culturally constructed, national styles	Actor network composed of scientists and non-scientists; configuration varies according to each "event"	Complex multifaceted (technological trajectories reversible according to social choice)	Emphasis in co-ordination and management. Accountability, maintenance of an independent scientific basis. Innovation policy	Peer review + direct public participation (emphasis given to the process), scenario building with ample social participation – foresight	Learning in a SI framework Co-ordination of donors, competitive funds for RTD

Source: Velho 2002.

conservation or management, of Central research institutes over State/ Provincial research stations. Institutions are the "working rules for going concerns"; institutions define organizations. (Bromley 1985) 'Organisations are formal structures with an explicit purpose and they are consciously created. They are players or actors.'(Edquist and Johnson 1997, p.47)

The distinction between organizations and institutions is important to understand the impact of agricultural R&D; both envisaged impact and actual impact. In the realm of innovations, broadly including technology generation and its impacts, institutions are necessary to cope with uncertainty, to manage conflicts and ensure co-operation among different research/ development actors, and to provide appropriate incentives for these actors (Edquist and Johnson 1997).

Institutions or rules may also be used to encourage or hamper certain lines of inquiry, and in yet another form can become entities that bring rigidity, allegations of 'institutional sclerosis', and the demand for progressive institutional reform (ibid.). Institutional economics classifies 'institutions' into hard and soft, formal and informal, mature and evolving; and 'institutional change' as driven by or preceding technological change, designed or self-grown, exogenous or endogenous, radical or incremental, moving towards a market equilibrium, a series of punctuated equilibria, or a perpetual non-consummatory evolution.

'If organizations are the players and institutions the rules, then how are the rules changed' (ibid. p.57)? It is crucial to bring the rules of the game, their physical organizations and the interplay of the two to the core of our questions about the way we do science and its interactions with the society that supports it. From a donor's perspective or more crucially, a farmer's perspective, how do we change some of the rules that govern our research and extension actors/ organization? How do we ensure that they learn from each other and take on board these lessons learnt into their decision-making processes? How do we ensure that cogent technological decisions consider both technological and institutional features?

The past two decades have highlighted the many drawbacks of the linear model of agricultural R&D, and brought a deeper understanding of innovation as a dynamic nonlinear process. This understanding of agricultural innovation systems is part of a learning process initiated by Biggs and other social scientists researching innovations and policy changes. In this genre of institutional understanding of innovations, the multiple sources of innovation model was the forerunner, emphasizing that research is never the exclusive source of an innovation (Biggs and Clay 1981). The contending coalitions and processes of negotiation that facilitate the generation and utilization of an innovation, and the iterative actor-oriented approaches and social capital that sustain the innovation process display the crucial role of institutional reform in successful

innovation systems (Biggs and Smith 1998, and Biggs and Matsaert 1999). In a successful innovation system, it is virtually impossible to demarcate the research and extension actors, both being involved with relevant stakeholders and combinations of technological and institutional innovations. The final blow to the linear model of R&D comes with the proof that innovations emerge as various actors adapt and adopt technologies, learning and changing the technology as they use it or exchange information about it with other actors/farmers (Douthwaite 2002). The stages and milestones in the conventional invention, innovation and diffusion process become redundant in a complex, adaptive multi-agent innovation system. The BNF community has a lot to learn from these social science insights into innovation and the impacts of innovation.

A proactive and socially relevant science does not in any way reduce the value of what science has already achieved, nor does it criticize the importance of discipline-based research and insights. It is important for us scientists to realize that our knowledge is not the sole source of BNF technologies or practices, and that our research has to undergo a de-centering to be located in a larger and wholesome but definitely more complex social context. Perhaps the right analogy is that of our own childhoods when we went through the phase of de-centering, and began our cognitive process of dealing with and negotiating our relationships with our own family, friends, and community. None of us would be the individuals we are today if this cognitive de-centering had not occurred. How do we accomplish this? We need to ask ourselves why the world of BNF technology generation, BNF extension and training, and BNF adoption are conceptualized as distinct units arranged in descending order. The institution or norm of hierarchy is explicit in the way research programs are conceptualized, and results transferred down to extension personnel, who then hand it down to farmers to adopt. Here, the need to learn, especially about the institutions and systems (actors/agents, their linkages or patterns of evolution) is minimal or nonexistent.

BNF Technology in the Linear Model

When perceived as an isolated technology component that can be introduced by external (trained extension) agents into a production system (farm or ecosystem), the BNF technology is cut off from the crucial institutional and social components of the system in which the technology works. This diffusion of innovation in a linear fashion, from the science that generates it to the extension effort that disseminates it down to the farmer who uses it, is the rule or institution that defines the organization of public sector research and extension.

Though the linear model has been widely discredited in the literature (Biggs 1991, Roling 1988, Nitsch 1994), it still holds sway, and the structural

and functional bifurcation of research and extension as two distinct organizations continues. Within the BNF community, this demarcation of functional boundaries for research and extension enables compartmentalized accountabilities. While researchers' roles are limited to characterization of rhizobial diversity, release of N_2-fixing legume varieties or identification of pathogens, the extension agent is only accountable for a certain number of farm visits or hours of training received from BNF experts or number of training sessions conducted for farmers. This compartmentalization ensures that scientific research does not face or acknowledge the complex processes of technology generation and use, and can continue to legitimately ignore the technological contexts (Hall and Clark 1995).

The linear model also ensures that both research and extension can blame each other for their own inadequacies and inefficiencies. BNF is rated as a successful technology that failed because of bad extension (Yanni 2002). Both research and extension accuse the policies that distort domestic or international pulse/oilseed prices, provide fertilizer subsidies, or succumb to political clout of (cereal/cotton/oilseed) lobbies. Whenever adoption rates are negligible, the linear model helps the policy-research-extension combine reproach the 'ignorant farmer' for failure to appreciate the fruits of technological change. The linear model, by ascribing direct causal linkages, does not allow introspection within these extension or research organizations. Why do some regions located within the same policy framework adopt the technology? In India, over 95% of the total All-India rhizobial inoculant (about 13,000 Mg in 2000-01) use in farming as well as production of inoculants take place in southern and western India (IISS 2001, Rao 2002).

Two of us (authors here) have been part of the extension system receiving packets of rhizobial inoculants, to be distributed much like we were asked to distribute targeted numbers of improved coconut seedlings, pesticides or small farm implements. The extension systems, with their centralized (State level) decision on targeted benefits and beneficiaries, divides the supply of inoculants by the number of districts to be covered under the State level BNF scheme. This district target is then divided into block and panchayat targets based on the cropped area. The concerned extension officers then distribute the inoculant packets to the farmer beneficiaries, with instructions (which are often delivered to the extension officers by the BNF subject matter specialists (SMS) at the State Agricultural University) on how to use the contents of the packet. The extension errors here are:

(1) Lack of awareness of local problems and the requisite research and communication support to solve them;

(2) Poor articulation of the problem of implementing centralized agricultural development programs, that are often irrelevant to the local context or needs;

(3) Treating dissemination of BNF technology like the dissemination of any other technology, say a new variety or farm implement developed in an agricultural research station – poor awareness of the biological and biophysical complexities associated with BNF;

(4) Little capacity to question or comment on the research system that does not learn from or address the variations in technological contexts at the farm level;

(5) Limited social science knowledge and analytical skills to address and implement systems based technologies like BNF.

It is therefore, a matter of convenience for research and extension that the adoption of BNF technologies is equated to number of packets of inoculants distributed, number of trainings received from the SMSs, number of training programs conducted at farm level, and number of farmer participants in these training programs. Extension, tuned to being evaluated in these 'numerical' terms, resigned to the hierarchy of research-led agricultural development, never challenges the assumption that number of packets of inoculants distributed is not the same as adoption of BNF-legume systems. Farmers often buy the inoculant packets to access credit and then never use the inoculants, because they had found that it never worked in their fields (Hall 1994, Hall and Clark 1995). Unless extension forcefully articulates the farmer's agenda, the complex technological contexts and the inherent biophysical complexity of BNF, the BNF bandwagon will keep on rolling. Extension's silence or its surrender without even engaging in the game has in no small measure contributed to the flawed decision-making and implementation of BNF programs and agricultural R&D programs in general.

The social sciences play a major role in legitimizing this compartmentalization of the science-technology-society continuum, and the linear model of agricultural R&D. The assumptions are that (1) there is a direct causal relationship between science and agricultural development, and (2) institutions do not matter or (at best) they are a direct function of relative factor scarcities (Raina 2001, Hall et al. 2001). The social sciences (agricultural economics in particular) are used to assess the impact of agricultural research (Horton 1998). This economic impact assessment then legitimizes the organization of agricultural research and extension. We would argue that approaches such as econometric estimation of their respective impacts, using proxy variables (research expenditure, publications, personnel, adoption rates, farmers education, income increases, etc.) are necessary for the political and economic legitimization of the linear model and increased funding for agricultural research. Little of the credit and funds go to extension.

Despite being invalidated in reality and discredited academically, the linear model survives because of two advantages. These are:

(1) the enhanced funding and legitimization through econometric estimation of returns to investment in (commodity-wise) research and (separately) extension, and

(2) the hierarchically justifiable scope for selective attribution of failures that are not counted in the quantitative measurement of successes.

Donors in the R&D scene now demand plausibility and proof of causal linkages, processes and negotiations that lead to successful innovation and agricultural development, instead of precise internal rates of return, lists of publications or varieties released (Herdt and Lynam 1992, Baur et al. 2001, Hall 2002). The persistence of poverty, unexpected and sometimes negative social and environmental consequences of technological change, and the evidence of new institutional arrangements in successful innovation systems often outside formal organized R&D has led to this demand for plausibility. Donors and decision-makers claim that the quantitative input-output analyses do not tell us about these new/ different institutions, capabilities, and actors in the innovation system. Therefore, the need for plausibility; these estimates offer no insights into the processes or capabilities that need to be built or placed to enable innovation and poverty reduction or social well-being (Baur et al. 2001).

How can the BNF research community establish this plausibility? How do we 'reposition N_2-fixing organisms and their products from a central auto-ecological focus into a more integrated component of a larger, more complex task?'(AABNF 2001, p. 13). How do we, natural and social scientists, extensionists, farmers, rural women, inoculant producers, and policy makers together face this complex task given our reluctance to face complexity? How do we break out of the convenient and simplistic linear models of innovation, and learn about the complex processes involved in innovation? It is a major challenge for the BNF innovation system to recognize and initiate these internal thinking and learning mechanisms.

INSTITUTIONAL LESSONS FROM THE LINEAR MODEL

Let us bring here a brief overview of the analysis and conclusions of a study on *Rhizobium* inoculants in Thailand (Hall and Clark 1995). The study explored the impact of *Rhizobium* inoculant technology on peasant agriculture. The objective however was more than mere estimation of benefits from the technology. The objective was to understand the processes of technology generation and diffusion, and the way these processes and actors influenced the adoption of as well as the gains from the inoculant technology. How did these technology generation and diffusion processes interact with, learn from, and change in response to different technological contexts? The study concluded that scientific research and extension components involved in

the inoculant technology did not learn from or change with the diversity of biological and social contexts they encountered (ibid.).

A brief (however inadequate) glance at the history of BNF technologies reveals that there are two important sources of impetus for BNF. The first and most renowned is the oil crisis of the 1970s and the consequent fear that the fossil fuel-dependent fertilizer industry may not be sustainable. The fear was enhanced by the food production-population growth rhetoric of the Green Revolution, thereby making it absolutely essential for experts and policy-makers to find and develop alternative sources of N fertilization for keeping the Green Revolution technology package going. Chemical fertilizers (besides assured irrigation and high-yielding varieties) were at the core of this technology package. This technology package had to be sustained, to ensure increased food production; in the face of a fertilizer industry threatened by fuel crisis, farms were to be fertilized by BNF.

The second and less popular is the history of BNF within organic agriculture, where all efforts to maintain soil fertility are natural or organic. Though it may be argued that the organic farming movement arose as an alternative to the mainstream chemical-based agriculture, millions of farmers in the poor predominantly agrarian economies have been practicing various forms of organic farming or combinations of organic and chemical-based agriculture. Here, the role of BNF is not a gap-filling assignment in the face of shortfalls in fertilizer supplies or increasing fertilizer prices. BNF has its legume-based place in the crop rotation or crop layout as demanded by specific agroecosystems and local economies. A crucial difference between BNF technologies in mainstream Green Revolution agriculture and in organic farming is that BNF is not a substitute for fertilizer but an integral part of the farming system in the latter. It is another process involving a series of internally sourced inputs, processes and impacts much like green manuring or biopesticides are.

Conceptually, the organic farming BNF technologies at the farm level are different from the Green Revolution BNF technologies. But at the level of BNF technology generation and development, both conventional Green Revolution and organic farming research frameworks view and operationalize BNF technologies as a strain of inoculants produced in the lab. These strains (sometimes with corresponding legume varieties experimentally proven ideal) are then transferred through extension agents to farmers as a packet of inoculants stored in the right conditions, purchased and used within the expiry date printed on the packet, and measured as units of yield increased due to inoculant application. This linear view of technology generation, diffusion and adoption is common to both mainstream Green Revolution agricultural science and the alternative equally rigid (pure) organic farming movement. Increasing disillusionment with both can be traced to the shared misconceptions and lack of awareness of farmers' knowledge and requirements. The organic agriculture movement is disillusioned with small

and marginal farmers using chemical fertilizer, though the practice is corroborated by the farmers' knowledge of both organic and inorganic systems and their benefits, short-term and long-term. See Gupta (2000) for an interdisciplinary analysis of decision-making on soil fertility.

Farmers are aware of a host of innovations that meet their demands – the demand for BNF may not be soil fertility alone. Farmers demand and do get other benefits from BNF – for instance, better seed germination and protection from insect pests and fungal pathogens (Hall and Clark 1995). These benefits and other impacts are highly location-specific. These location-specific features of agriculture, such as farmers' knowledge or cultural (food/ culinary) preferences, traditional pest control systems, edaphic or land use histories or seasonal variations are not features that can be resolved statistically using multi-location trials and control plots. They demand a broader understanding of agriculture and its knowledge systems – formal (professional), industrial (manufacturing applications), local and farmer-based (socioeconomic and biophysical expectations/ impacts).

The linear view of BNF technologies precludes this systems perspective of BNF as a part of a larger innovation system. Table 2 presents some of the technological challenges that the BNF research community addresses and some of the institutional and social challenges that are handled by the extension/transfer of technology networks. The latter are physically and conceptually located outside the research effort and get to address the BNF technologies after the research organizations/programs generate these technologies. Table 2 reveals that much of the organized scientific world is chaotic too. There are disciplinary boundaries, organizational mandates and selective perceptions that distort the direction and content of scientific research from the real problems in soil fertility management or ecological sustainability that BNF can address.

Our existing BNF R&D systems make a clear logical distinction between the technology (BNF/legume variety/crop rotation/prescribed management practices) and the social processes or contexts that facilitate the utilization of the technology. The role and responsibility of science ends with the generation of the technology – in this case often limited to the inoculant strain. This is a legacy of the conventional instrumental view of science that makes a logical distinction between reality and morality (Hagedorn 1993, p. 859). In the practice of science, this distinction is guaranteed by an assumed division of labor between scientists and politicians, where the sole responsibility of science is to discuss "means on the basis of given objectives" (op cit). Once the technology has been generated, the extension agent takes over to mediate the transfer of the technology from the organized logic of the scientific world to the complexities of the real world.

How can a 'research project' on enhancement of N_2 fixation (see row 7 in Table 2) be formulated and implemented without acknowledging and

including the crucial information about local management practices and farmers' expectations, which are conventionally slotted into the extension box? How can geneticists and plant breeders produce N_2-fixing legume varieties (see row 2 in Table 2) without the active participation of social scientists (economists/sociologists/extensionists) in identifying and selecting crucial varietal traits that may not meet market demands or correspond to the farmers' decision criteria? The geneticists and molecular biologists who made some excellent molecular discoveries in the late 1980s have moved on and left the BNF technology arena where it was before. The appropriate networks or clusters of actors to take these molecular discoveries forward to commercialization and utilization in the fields did not emerge in the 1980s because there was no scope for an innovation system to emerge within the prevailing linear model. We argue here that these geneticists and molecular biologists were not part of a larger innovation system with appropriate uptake pathways, non-hierarchical interdisciplinary discourses and stakeholder participation (policy makers/ farmers' groups). It is important that we understand past research directions, opportunities missed and created, in order to figure out how we can change or reconfigure the conventional technology generation, diffusion, adoption compartments into socially relevant innovation systems.

Conventional BNF programs are located within the disciplinary constructs of microbiology or soil chemistry. Plant breeders are often reluctant or skeptical to take up crop improvement projects to select varieties for N_2-fixing traits (Hall and Clark 1995). BNF research conforms to the disciplinary hierarchy in the 'hard science culture' of agricultural research organizations. Organizationally, agricultural science and the assessment of its impact on society have been governed by the norms of the natural sciences. (Horton 1998). Plant breeders do conduct or collaborate in the conduct of trials of nodulation/ N_2-fixing capacity in some of the varieties they have developed. But these trials are often conducted within the hierarchy of the agricultural science disciplines, maintaining the superiority of plant breeding. Similar professional differences between soil chemists and agronomists have also been discussed within the BNF community (Yanni 2002, Raina 2003).

Table 2 summarizes the criticisms of or shortfalls in BNF R&D programs, gleaned from several sources and discussions. Scientists' claim that each technological challenge listed here has been solved or can be solved by science. But they claim that corresponding challenges in other disciplinary domains, and social/institutional challenges inside and outside the laboratory or experiment station are not in the realm of their discourse. Accordingly, these challenges must be handled by the nonscientific (technology transfer/service sector) professionals and the local farming community or its political representatives. But some of these allegedly external challenges are located within or originate from the scientific discourse and cannot be solved without

Table 2. Conventional BNF R&D - technological and institutional realms.

S. No.	Technological problems/ realms addressed	A few related institutional or social challenges
1.	Characterization/isolation / screening biodiversity / inoculants	a. Local biological diversity/rhizobia b. Soil microbiology vs. soil chemistry vs. agronomy
2.	N_2-fixing legume varieties	a. Entry legumes/replacements b. Farm-level decision criteria for crop choice c. Reluctant plant breeders d. Inadequate extension feedback e. Market demand
3.	Host specificity	a. Farmers' crop rotations/layouts b. Competition with local rhizobial strains c. Soil/land management patterns
4.	Transfer/ expression of nif genes	a. Genomics vs. plant breeding hierarchies/disciplinary barriers b. Differing crop physiological requirements (fodder/dry matter /fuel) c. Radical green movements
5.	Fertilizer combinations (especially P)	a. Nature of enterprise (crop/crop-livestock/agro-silvicultural, etc.) b. Seasonal variations – household and crop response to stress c. Market price/availability/access d. Fertilizer policy/subsidy
6.	Ideal soil properties	a. Local classification, edaphic history b. Disciplinary boundaries (agronomy vs. soil chemistry) c. Attitudes of farmers, policy-makers, industry.
7.	Enhancement of N_2 fixation under multiple/ mixed cropping and residual fertility effects	a. Generalization or specificities across zones/crops/production systems/environments b. Local management practices and farmers' expectations c. Micro and meso environments – effects on physiological factors, photoperiod response, etc.

Contd.

Table 2. Contd.

S. No.	Technological problems/ realms addressed	Institutional or social constraints/challenges faced
		d. N_2 uptake vs. N_2 fixation – farmers knowledge and choices e. Cultures and philosophies of stewardship or sustainability
8.	Farmer awareness/ resource constraints	a. Choices within vs. choices across constraints – perceptions limited to 'economics' b. Policies, politics, and production systems constraints c. Farmer's decision-making criteria d. Gender, labor, nutritional, cultural conditioning
9.	Inoculant technology development and transfer	a. Training of extension staff b. Inoculant production, processing and storage facilities c. Links with industry d. Quality assurance/standards e. Market price of legumes/pulses, other inputs costs f. Credit and other services g. Fertilizer policy and the overarching production-oriented agricultural policy, favoring favorable production tracts and well-endowed farmers.
10	Biosafety assessments	a. Biosafety regulations/organizational disjunctures b. National environmental policy c. Livelihood strategies – especially in marginal/fragile ecosystems d. EIA – practices, values and norms

the active participation of scientists and their disciplines/methods. It is now recognized that the BNF science community can no longer be "immune to larger social and environmental issues." They cannot expect "others to recognize and translate their research products into forms applicable to them." (AABNF 2001, p.9) The BNF research community therefore needs to recognize that BNF research must not be conducted for its own sake, but rather should be regarded in a broader innovation systems context (ibid.). The twenty-first century paradigm for BNF impacts, according to the AABNF is:

> Research in biological nitrogen fixation must be nested into larger understanding of system nitrogen dynamics and land management goals before the comparative benefits of N_2 fixation may be realistically appraised and understood by society as a whole.
>
> (AABNF 2001, p. 12)

The innovation systems perspective evident here makes equal demands from professional actors and society in general, to enable the nesting of BNF into system dynamics and land management goals. There are rules of the game or institutions of science that must change. These institutional changes will also include changes in the relationships among and capacities of crucial organizations of research funding, research conduct, extension and input delivery, legume marketing and processing, and policy making.

CONCLUSION: LEARNING TO CHANGE

In conclusion, it is important for us to see that the AABNF, whose members are scientists, have arrived at a new paradigm of BNF research. This AABNF paradigm is the antithesis of the conventional model of socially and ecologically isolated science. It is also the antithesis of the linear model of research and development. It is the nonlinear, innovation systems paradigm. None of the professionals who crafted this new AABNF paradigm were aware of the innovation systems paradigm or the institutional/evolutionary economics theories that led to it. This is a case of experiential learning and institutional change that ensues from this learning.

For the AABNF, shifting from the linear model to this innovation systems paradigm was not easy. There were major disappointments with the way BNF technology was being generated and transferred to the apparently natural exclusion of 'larger social and environmental issues.' Experts within disciplines like microbiology who had been exposed to systems thinking were increasingly frustrated at the linear thinking prevalent in BNF research. Pressures from the donor community as well as evident poverty and resource degradation in the African countries demanded a re-think from within science. That this thinking mechanism triggered further questions about the policy context of BNF research proved to many within the BNF research

community the need to locate BNF research within 'larger social and environmental understandings.' Yet, some of the earlier rethinking (during the early-mid 1990s) within the AABNF remained unanchored ideas, at best among two or three scientists. Putting these ideas about a new paradigm on paper and discussions among the AABNF community took several years. Building a community of practice convinced about the need for change was a major achievement. It was a unique combination of microbiologists and systems agronomists (working on alley farming) that made this dialogue and learning possible. The role of systems perspectives and the impossibility of making relevant impacts with excellent but isolated research became evident in several rounds of discussions among these alley cropping systems researchers and microbiologists.

Yet, conviction about the need for these larger understandings or the prospect of better opportunities in achieving better impact through an innovation systems perspective in BNF research is still not a common attribute applicable to all AABNF members. There are many that still are convinced that all scientific research in the domain of BNF-legume symbiosis is perfect. Some still believe that scientists have little to do with all the problems due to inadequate extension, improper storage facilities, bad policy frameworks, etc. The old guard of linear compartmentalized research and development models will pose a continuous pressure to return to status quo. But the innovation systems perspectives of issue-based R&D networks like the AABNF are unlikely to forget the lessons they have learnt or the innovation systems perspective they have identified as central to successful BNF innovations.

The case of the AABNF highlights the conclusions emerging from this paper. For successful BNF technologies, we need to recognize that there is a range of actors, researchers and non-researchers, involved in the innovation process. These actors see the entire innovation system as a process, and not as compartmentalized hierarchies, where the technology is handed down from one level to the next lower level. In the ideal BNF innovation system, this will involve continuous learning; shifting roles for technology/information producers and information users, and a need-based exchange of knowledge (Lundvall 1992). This places great emphasis on communication and learning skills within scientific research and extension systems as well as in society. It also demands that research managers or policymakers identify and deliberate a range of incentives, capabilities and facilitation processes that might enable several diverse actors to come together and to evolve together as part of the innovation system. The innovation systems approach calls for a broader understanding of agriculture and its knowledge systems – formal (professional), industrial (manufacturing applications), local and farmer-based (socioeconomic needs/constraints and biophysical expectations/impacts). It challenges the existing norms or institutions of agricultural

research and development. It demands new arrangements and combinations of technical and institutional innovations.

BNF is more than a single variety or input or manurial practice. It is a technology that straddles several arenas of knowledge; the biological, chemical and physical aspects of soil and water systems and its interactions with the crop/farm, and the historical, social and institutional contexts of all the actors involved. Understanding the complex technological and institutional contexts and responding to their specificities is an internal competency that science needs to develop if it is to be part of an effective innovation system. Internal learning mechanisms are crucial in a BNF innovation system. Involvement of social scientists (not limited to economists) and natural scientists, policy makers, extensionists, input producers/distributors, and farmers to favor BNF-legume components in their larger technological and social systems places a significant pressure for internal learning on formal agricultural research organizations and its scientists. There are significant lessons from ICRISAT, teaching us how scientists can be core actors in an innovation system, can work most efficiently as part of a larger network, learning and sharing responsibilities, resources and credit (Reddy et al. 2001).

While learning is a requirement, what we need to prepare ourselves for is a great deal of unlearning. Agricultural science in general and the BNF community in particular need to unlearn a few crucial institutions or norms of agricultural research and development. One of the first institutions is the assumption that agricultural technology leads to specific impacts and thereby to agricultural development. We need to unlearn this technological determinism. With every successful technology, there is a range of institutions, rules or relationships that evolved and enabled the successful application or propagation of that technology. Another unlearning imperative has been discussed repeatedly in this paper. It is the implicit hierarchy in the agricultural research and development organizations. We need to transcend this notion of the superiority of science over other knowledge systems, especially extension or farmer's knowledge. Yet again, attribution of benefits or impacts to a single agency, i.e., research, demands unlearning. The history of the Green Revolution has proved that subsidies (and increasing profits) to the fertilizer industry, ample investment in public irrigation works, conscious policy choices about regions/districts of focus and several other institutional changes accompanied the high yielding varieties-chemicals technology package. The BNF innovation system enables us to see that it is not BNF technology alone that brings social and economic benefits. The ability of BNF to contribute to social and economic needs depends on the products and processes realized from other partners or components (actors or agencies ranging from research or extension organizations, farms and ecosystems, private firms, government department, production and processing units,

etc.) of the innovation system. We have to unlearn the assumption that all benefits from technological change can be attributed exclusively to agricultural science.

With a larger systems vision and practice, BNF innovation systems can help the entire body of agricultural science, technology generation, diffusion and adoption make the transition from linear R&D models to nonlinear systems of innovation. The innovation systems approach does not negate the important achievements in science or the need for more scientific research, especially discipline-based research. On the contrary, an innovation systems perspective gives us the basic competency we need to see the systems components and relationships that enabled the success of our technologies or the failure of others. A BNF innovation system involving technological and institutional change can make an impact on agricultural development and poverty reduction.

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Rhizobium Inoculation of Leguminous Oilseeds – Results of On-Farm and Farmers' Field Demonstrations in the ICAR Coordinated Project on BNF

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ABSTRACT

BNF research and applications in India are nearly nine decades old, but have received greater attention only during the last four decades. The AICRP on BNF was established in 1978 for intensifying BNF research all over the country. BNF technologies have been successfully demonstrated on farmers' fields in front-line demonstrations in three Indian states. Additional groundnut pod yields due to *Rhizobium* inoculation in farmers' fields were 246-424 kg ha^{-1} in red sandy loams in Tamil Nadu and 263-302 kg ha^{-1} in clay to clay loam Vertisols of Maharashtra. Soybean seed yield increase due to inoculation ranged from 80-160 kg ha^{-1} in clay loam Vertisols of Madhya Pradesh. Co-inoculation of rhizobia with plant growth promoting rhizobacteria (*Pseudomonas*) increased groundnut pod yields by 6-12% over rhizobia alone. Application of farmyard manure @ 5 Mg ha^{-1} along with *Rhizobium* inoculation significantly increased groundnut yields in Vertisols. Rotational benefits of soybean inoculation on succeeding wheat crop were ~30 kg N ha^{-1}.

INTRODUCTION

Biological nitrogen fixation (BNF) plays an important role in maintaining the fertility of the low-nitrogen (N) containing soils of the semi-arid tropics.

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It is now increasingly being realized that integrated plant nutrient systems (IPNS) involving a combination of fertilizers, organic/green manures and microbial inoculants are imperative to sustain crop production and maintain soil health and soil biodiversity in the long run (Wani et al. 1995). This is especially important for developing countries where farming will continue to be in the hands of small farmers. In a country like India, the demand for nitrogen fertilizer is expected to go up from the present level of 11.4 million t (in 2001-02) to 13.9 million t by 2006-07 and 16.2 million t by 2011-2012 AD. The economic burden and environmental cost of applying such a high quantity of additional fertilizer is obvious. Even if a part of this increase in the demand for N can be met from BNF, the likely savings will be enormous.

Research and applications of BNF in India are nearly nine decades old but have received greater attention during the past four decades. The ICAR's All India Coordinated Research Project (AICRP) on BNF began as an ICAR ad hoc scheme in 1976, in response to the oil crisis which had caused a steep hike in the N fertilizer prices. It was approved as a regular plan scheme in April 1978 in the sixth Five Year Plan to intensify BNF research throughout the country. Due to the concerted efforts of various organizations and departments, various microbial inoculants for all crops in different agroecological regions of India have become available for augmenting the supply of mainly N and P in various cropping systems and for promoting plant growth in general. This paper focuses on the use of microbial inoculants for leguminous oilseeds.

BIOLOGICAL NITROGEN FIXATION IN OILSEED CROPS

Oilseed crops are cultivated on about 24.4 million ha in India (1999-2000), of which soybean (*Glycine max* L.) occupies 6.0 and groundnut (*Arachis hypogaea* L.) 6.9 million ha. These account for 12.1 million t of edible oil production out of a total of 20.9 million t (Hegde 2002). Soybean has been intensively cultivated since the 1970s in central and western India, while groundnut is principally grown in western and peninsular India. Because of the specificity of symbiosis, soybean bradyrhizobial strains were initially imported from USA. Groundnut is nodulated by many rhizobia and bradyrhizobia of the tropics and subtropics. In a farmers' field survey, nodulation in groundnut was found to be inadequate in more than 50% fields and even where adequate, ineffective nodules exceeded effective ones (Nambiar et al. 1982). The identification and inoculation of competitive, efficiently nodulating, nitrogen-fixing strains of rhizobia could solve the problem of ineffective nodulation by native rhizobia. Inoculation with effective *Rhizobium* strains increased groundnut yields in several field experiments in India (Sundara Rao 1971). Due to low organic matter content of tropical soils and high temperatures in the summer the rhizobial populations are low and hence there is a need to

build up populations of the desired strains by repeated inoculation as well as addition of organic materials. Beneficial free-living soil bacteria are usually referred to as plant growth-promoting rhizobacteria (PGPR), for example *Azospirillum* spp., *Pseudomonas* spp. and *Bacillus* spp. It has been known for many years that co-inoculation of PGPR like *Azospirillum* along with *Bradyrhizobium* promotes nodulation and BNF in soybean under controlled conditions (Singh and Subbarao 1979), but field studies are few. Besides the need to demonstrate to farmers the usefulness of inoculating rhizobia for legumes, there is also a need to quantify the savings in fertilizer nitrogen for the rotational crops, grown in sequence, in order to convince the farmers of the utility of legume inoculation and effectively promote BNF technologies. The present paper summarizes recent results from selected on-station trials and farmers field demonstrations on soybean and groundnut conducted by AICRP on BNF in three Indian states on (1) Frontline demonstrations on *Rhizobium* inoculation (2) Co-inoculation of rhizobia with PGPR (3) Role of organic amendments in improving legume yield and (4) Rotational benefits of soybean growth and of *Rhizobium* inoculation on the succeeding wheat crop.

Front Line Demonstrations on Oilseeds – Effect of *Rhizobium* Inoculation

Under a Ministry of Agriculture sponsored scheme (Front Line Demonstrations (FLD) on Oilseeds–subcomponent D: Effect of *Rhizobium* inoculation), demonstrations were carried out in farmers' fields on one-acre plots (½ acre control, ½ acre inoculated) in three Indian states, Tamil Nadu, Maharashtra and Madhya Pradesh. The details of the experimental sites and soil properties are given in Table 1. The pH in 1:2 soil-water suspension, organic C by Walkley-Black wet digestion method, available N by alkaline permanganate procedure, available P by Olsen's method, and available K in neutral ammonium acetate soil extracts were determined as per procedures described in Hesse (1971). The recommended levels of fertilizers, NPK and plant protection chemicals were used in all the field trials.

In red-sandy loam soils in Coimbatore district (Tamil Nadu, South India), 19 FLDs were carried out on soybean during 1995-2000. Increases in seed yield ranged from 12.6 to 17.9% due to inoculation, with absolute grain yield benefits ranging from 155 to 289 kg ha^{-1}. (Table 2). In groundnut, 27 FLDs were conducted in Coimbatore district; pod yield increase ranged from 14.7 to 23.4 %, with absolute pod yield benefits in the range of 246-424 kg ha^{-1}. In clay loam soils (Vertisols or black soils) in Parbhani district (Maharashtra, Western India), yield response was in the range of 23-28% increase in groundnut pod yield due to inoculation (Table 2) in varieties Tag-24 and SB-XI, with absolute pod yield increase ranging from 263 to 302 kg ha^{-1}. These effects are similar to the responses to inoculation with effective strains

Table 1. Soil properties of the experimental sites of front line demonstrations on *Rhizobium* inoculation on soybean and groundnut.

Site	Aliyarnagar	Bhavanisagar	Paiyur	Parbhani	Jabalpur
State	Tamil Nadu	Tamil Nadu	Tamil Nadu	Maharashtra	Madhya Pradesh
Texture	Red sandy loam	Red sandy loam	Loamy sand	Clay to clay loam	Clay loam
pH (1:2)	7.1	7.2	7.5-8.0	8.1	7.0
Organic C (%)	–0.35	0.34	0.32	0.47	0.62
$KMnO_4$ oxidizable N (kg ha^{-1})	255	170	230	250	186
Available P (kg ha^{-1})	10.8	19.0	11.0	10.0	11.7
Available K (kg ha^{-1})	230	210	250	220	290

Table 2. Front line demonstrations in farmers' fields on inoculation of *Rhizobium* in groundnut and soybean.

Site (District)	State	Years	Crop	No. of trials	Pod/Seed yield (kg ha^{-1}) Control	Inoculated	% increase
Coimbatore	Tamil Nadu	1995-2000	Groundnut	27	1467-1811	1713-2235	14.7-23.4
Parbhani	Maharashtra	1995-2000	Groundnut	16	1045-1150	1308-1419	23.1-28.1
Jabalpur	Madhya Pradesh	1999-2000	Soybean	5	480-1280	560-1400	9.4-16.7
Coimbatore	Tamil Nadu	1995-2000	Soybean	19	1139-1618	1294-1907	12.6-17.9

reported by others. For example, inoculation of an effective strain NC 92 significantly increased the yield of groundnut cultivars Robut 33-1 at ICRISAT centre, Patancheru (Nambiar et al. 1984) and cultivar JL24 at Junagadh (Joshi and Kulkarni 1983). Two groundnut *Rhizobium* isolates IGR6 and IGR40 (National Research Centre for Groundnut, Junagadh) enhanced the pod yield of groundnut by 11 to 18% at multiple locations over the years (Pal et al. 2000).

In clay loam soils (Vertisols or black soils) of Jabalpur (Madhya Pradesh, Central India), three years' field data of on-farm trials summarized by Rao (2001) showed that a 1.8 t ha^{-1} seed harvest of soybean crop removed 187 kg N ha^{-1} of which BNF component was ~150 kg ha^{-1}. *Bradyrhizobium* inoculation consistently increased seed yields by 240-390 kg ha^{-1}. A similar range of increase in soybean seed yields due to inoculation (230-410 kg ha^{-1}) was observed in a Vertisol at Sehore, Madhya Pradesh (Dubey 1998). Nitrogen fixation ranged from 76.1 to 137.6 kg ha^{-1}. In the present study five farmers' field demonstrations of *Bradyrhizobium* inoculation on soybean cultivar JS-335 were carried out in 1999-2000. Averaged over all the five sites, nodule numbers increased from 15 nodules per plant in control to 28 nodules per plant in inoculated plots, nodule mass increased from 21 mg per plant to 42 mg per plant due to inoculation (data not shown). *Bradyrhizobium* inoculation resulted in increase in the yield (9.4-16.7%) of soybean at all the five locations. The mean yield was 880 kg ha^{-1} under noninoculated conditions, while it was 990 kg ha^{-1} after inoculation (Table 2). In this study a mixture of 3-4 indigenous strains of *Bradyrhizobium* isolated from Jabalpur soils were used. With *Rhizobium* culture treatment, the farmer gained an increment of 110 kg ha^{-1} (range 80-160 kg ha^{-1}) in soybean seed yield. By spending a small amount of Rs. 20.00 only (0.40 US $), the farmer was benefited by Rs. 886.00 over recommended practices (selling price of soybean @ Rs 8000 per ton) per hectare. The benefit to cost ratio was thus 44.

Co-inoculation of *Rhizobium/Bradyrhizobium* with PGPR on Groundnut

In an on-farm field trial at Aliyarnagar in Coimbatore district of Tamil Nadu, inoculation of *Bradyrhizobium* Tt 9 along with *Pseudomonas* PS 2, a strain of PGPR, at 100% N and P application level gave maximum pod yield (Table 3). Inoculation saved 25% N and P; co-inoculation of *Pseudomonas* was significantly better than inoculation with rhizobia alone, particularly at 75% N and P level.

To further confirm the above effects, multi-locational trials were performed at three agricultural research stations in Tamil Nadu on combined inoculation of slow growing *Bradyrhizobium* Tt9 and PGPR *Pseudomonas*. Groundnut pod yield increases due to co-inoculation over *Bradyrhizobium* inoculation

Table 3. Effect of combined inoculation of *Rhizobium/Bradyrhizobium* and *Pseudomonas* on groundnut.

Treatment	Nodule (nod pl^{-1})	Nodule DW (mg pl^{-1})	Pod yield (kg ha^{-1})	% increase in pod yield over control
100% N and P				
Noninoculated control	20	120	1333	—
Rhizobium (TNAU 14)	43	160	1399	5.0
Bradyrhizobium (Tt9)	42	170	1433	7.5
Pseudomonas (PS2)	33	160	1415	6.2
TNAU 14+ PS2	47	220	1492	11.9
Tt9 + PS2	44	180	1517	13.8
75% N and P				
Noninoculated control	21	100	1001	—
TNAU 14	33	160	1042	4.1
Tt 9	36	210	1083	8.2
PS2	29	160	1024	2.3
TNAU 14 + PS2	39	270	1278	27.6
Tt 9 + PS2	38	190	1351	34.9
L.S.D. (p=0.05)	3	35	69	—

Table 4. Combined inoculation of *Bradyrhizobium* (Tt9) with PGPR *Pseudomonas* on groundnut at different locations in Tamil Nadu.

Treatment	Pod yield (kg ha^{-1})			
	Bhavanisagar		Paiyur	Aliyarnagar
	1998	1999	1999	1999
Non-inoculated	1462	1333	916	2139
Bradyrhizobium	1596	1433	1083	2443
Bradyrhizobium + *Pseudomonas*	1739 (9.0)*	1517 (5.9)	1166 (7.7)	2763 (12.3)

*Percent increase in yield over bradyrhizobial inoculation

alone were obtained at all three locations (Aliyarnagar, Bhavanisagar and Paiyur) and ranged from 6 to 12% (Table 4).

Plant growth promoting rhizobacteria are known to improve N_2 fixation by increasing the number of most active nodules (greater acetylene reduction activity) and increased effectiveness (more leghaemoglobin) (Groppa et al. 1998), greater proportion of N derived from fixation (Galal 1997), solubilization of fixed forms of phosphates, production of phytohormones like IAA and gibberellins (Molla et al. 2001), production of siderophores for chelating iron (Pal et al. 2000), and synthesis of low molecular weight compounds or enzymes that can modulate plant growth and development. PGPR are also reported to produce antibiotics that suppress deleterious rhizobacteria /plant pathogenic fungi or through other unidentified mechanisms, all of which provide a healthy environment for better root growth.

Role of Organic Amendments in Improving Legume Yield

In traditional soybean-growing areas in the Vertisols of central India, populations of soybean rhizobia were <100 cells g^{-1} soil in the 0-15 cm soil during summer due to prevailing high temperatures and remained at <100 cells even post-monsoon (Raverkar et al., unpublished). Similarly, the population of groundnut rhizobia was below 500 cells g^{-1} in the 0-15 cm and <100 cells g^{-1} in the 15-30 cm soil layer in red sandy loam soils of Tamil Nadu in the soils of the FLDs described earlier. The rhizobial populations in subtropical soils are thus well below the threshold for optimum nodulation and reinforce the need for repeated rhizobial inoculation each year to build up the populations, as well as application of organic materials like farm yard manure (FYM) to increase soil organic carbon content.

A three year study (1994-95, 95-96 and 96-97) was carried out in a clay loam Vertisol at Parbhani, Maharashtra on application of FYM @ 5 Mg ha^{-1} in a field experiment on *Rhizobium* inoculation of groundnut grown with recommended dose of NPK (25:50:30 kg ha^{-1}). Nodulation and nutrient concentration was determined at 60 days growth. *Rhizobium* inoculation increased the pod yield by 390 kg ha^{-1} while application of FYM alone @ 5 Mg ha^{-1} increased the yield by 150 kg ha^{-1} (Table 5). Combined application of FYM and *Rhizobium* increased the yield by 730 kg ha^{-1}. Nodulation and N and P uptake at 60 days and *Rhizobium* population in soil were all boosted due to combined application of FYM and *Rhizobium*. These and similar results in pigeonpea and green gram led to the recommendation from AICRP on BNF at Parbhani to 'Apply *Rhizobium* inoculants along with FYM @ 5 Mg ha^{-1}'. Addition of FYM is known to boost microbial activity and rhizobial proliferation, which improves BNF in legumes. Ndfa (nitrogen derived from air) in soybean improved from 46.1% in control to 62.5% at 4 Mg FYM ha^{-1} (Kundu et al. 1998). Rani and Sanoria (2000) showed beneficial effects of inoculation of *Bradyrhizobium japonicum* (USDA 110) on soybean nodulation, BNF and nutrient uptake, which were further boosted by addition of cattle dung manure or digested sludge @ 5 Mg ha^{-1}.

Table 5. Effect of build-up of rhizobia by organic amendments on yield of groundnut JL 24 in a Vertisol (1996-1997, average of 3 years' data, Parbhani).

Treatments	Pod yield (kg ha^{-1})	No. of nod pl^{-1}	Nod. wt mg pl^{-1}	% shoot N at 60 d	% shoot P at 60 d	% P in pods	*Rhizobium* x 103 g^{-1} soil
Control	1000	53	41	1.22	0.16	0.19	12.1
Rhizobium	1390	71	60	1.36	0.25	0.29	68.3
FYM @ 5 Mg/ha	1150	58	48	1.29	0.23	0.28	26.5
FYM + *Rhizobium*	1730	100	76	1.63	0.30	0.33	83.8
LSD (p=0.05)	160	3	-	0.10	0.11	0.10	—

BENEFITS OF SOYBEAN ROTATION ON SUCCEEDING WHEAT CROP

Benefits of legume rotation on succeeding cereal crops are well known. Two years of on-farm trials in a Vertisol at Jabalpur showed that wheat yields in soybean-wheat sequence were 11.8% higher than in sorghum-wheat sequence (Table 6); the nitrogen credit due to *Bradyrhizobium* inoculation of soybean

Table 6. Nitrogen economy through *Bradyrhizobium* inoculation in soybean-wheat cropping system (yield of wheat, Mg ha^{-1}) at Jabalpur.

Treatment N (kg ha^{-1})	Soybean-wheat (Mg ha^{-1})		Fallow-wheat (Mg ha^{-1})		Mean
	1998	1999	1998	1999	
Control	3.08	3.54	1.58	3.81	3.00
30 N	3.91	3.81	2.37	3.67	3.44
60 N	4.54	4.88	3.25	3.28	3.99
90 N	4.64	4.33	3.77	3.78	4.13
120 N (-Inoc.)	4.81	4.90	3.77	3.30	4.20
120 N (+Inoc.)	4.79	5.19	3.79	4.56	4.58
Mean	4.30	4.44	3.09	3.73	—

LSD p=0.05	Crop		Nitrogen		Crop x Nitrogen	
	1998	1999	1998	1999	1998	1999
	0.09	NS	0.15	NS	0.21	NS

was ~30 kg/ha. Benefits of soybean on succeeding wheat crop due to residual nitrogen were found to be in the range of 0.22-0.57 Mg ha^{-1} (Dubey and Srivastava 1991) in a Vertisol at Sehore in Madhya Pradesh.

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The Role of Biological Nitrogen Fixation in Increasing Crop Production and Soil Fertility in Vietnam

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ABSTRACT

BNF has a positive effect on growth and yield of most agricultural crops and on soil in Vietnam. The field test of BNF inoculants showed that rhizobial inoculants increased grain yield of groundnut 13.8%-17.5% in North and Central Vietnam and 22% in South Vietnam. Depending on the fertilizer status and nutrition content of soil, BNF inoculants increased rice yield by 4.07-19.59%, tea by 9.1-26.7% and maize by 9.4-10.2%. In addition, inoculants improved plant resistance to some pathogen diseases. Mixed culture of nitrogen fixing, phosphate solubilizing, and plant growth promoting microorganisms had a positive effect on growth and yield of different crops and increased profits. It increased the yield of rice 15.2-15.7%, and of soybean 16.3-19.5%. BNF also has a positive effect on the growth of forestry trees. The paper discusses various constraints of BNF application in Vietnam, and possible solutions to these.

INTRODUCTION

Vietnam is predominantly an agricultural country with the land under agriculture expanding at the rate of about 235,200 ha per year. Thus, cultivated area increased from 9.04 million ha in 1990 to 12.5 million ha in 2000. The demand for fertilizer is high and ever increasing. For the past 15 years, every year the mineral fertilizer supply has been continuously increasing by 7% for nitrogen-, 8% for phosphorus- and 12% for potassium-based fertilizer, and this trend is likely to continue in the future (Vu Nang Dung 2002). At present, the chemical fertilizer factories in Vietnam can meet only 20% of N- and 80% of P-fertilizer demand (Nguyen Van Bo 2001).

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The government has to balance this shortage by imports, which are very costly (nearly $500 million per year) (Tu Kien 2000). To improve this situation, Vietnam is trying to stimulate the research, production and utilization of biofertilizers, including biological nitrogen fixation (BNF) inoculants. Various BNF inoculants have been developed in Vietnam. Sterile or nonsterile carrier-based and liquid inoculants are produced on a small scale and applied by different methods. The N_2-fixing inoculants are as effective as application of 30-60 kg mineral N ha^{-1} yr^{-1} and can increase the legumes crop yield from 5% to 25%. In rice, maize and vegetable cultivation, N_2-fixing inoculants improve both the crop yield and resistance to some root diseases. Inoculants are produced from single strains like *Rhizobium, Azotobacter and Azospirillum* and also from the mixed culture of N_2-fixing, phosphate-solubilizing and plant growth-promoting microorganisms for different crops in different ecosystems. In other words, BNF is being applied for soil fertility improvement and soil erosion control by different legume species. This paper discusses the use of BNF in improving crop production and soil fertility in Vietnam.

THE USE OF BNF IN INCREASING CROP PRODUCTION

Various BNF-inoculants have been developed in Vietnam for application in increasing crop production. These include *Rhizobium* inoculant for legumes (such as soybean, groundnut and mungbean), free-living or associate N_2-fixing inoculant for rice, maize, vegetables and forestry plants, or inoculant from a mix of N_2-fixing and P-solubilizing microorganisms. BNF-inoculants are produced in peat carrier-based or liquid formulation. In recent years, Vietnam scientists have reviewed experiences and progress in inoculant production technologies from countries like Russia, Australia, India and USA, and developed simple fermentors for multiplication of microorganisms

Table 1. Effect of *Rhizobium* inoculant on groundnut yield.

Soil/cropping system	Fertilizer	Grain yield ($t.ha^{-1}$)		%increase over control
		Control	Inoculated	
Infertile soil	NPK:30:60:60, FYM 5t	1.97	2.27	15.2
Fertile soil	NPK:30:60:60, FYM 5t	2.31	2.63	13.8
Feralit soil	NPK:30:60:60, FYM 5t	1.58	1.85	17.5
New cultivation	NPK:30:60:60, FYM: 5t, Lime:5t	1.56	1.78	14.0
Intercropped Rice-groundnut	NPK:30:60:60 FYM: 5t, Lime:5t	1.50	1.66	31.0
Intercropped Vegetable-groundnut	100 kg ammonium sulfate (SA), 70 kg KCl, 150 kg coconut ash	1.41	1.69	20.0

Source: Ngo The Dan et al. 2000

in local conditions. The effects of different inoculants when tested in the field are presented in Tables 1 through 7. The field test dates showed that rhizobial inoculant could increase grain yield of groundnut by 13.8-17.5% in North and Central Vietnam and 22% in South Vietnam.

The tests showed that using BNF-inoculant is as effective as application of 30-60 N ha^{-1} yr^{-1}. This effect can be clearly seen in infertile soil and in legumes grown on newly cultivated land. Use of *Rhizobium* inoculant can give profits of 442,000 Vietnam Dong (VND) ha^{-1} in North and Central

Table 2. Effect of *Rhizobium* inoculant on soybean yield in the Mekong Delta.

Treatment	Pod yield (t ha^{-1})	Fertilizer input (In 1000 VND)	Input	Output	Profit
			(In 1000 VND)		
0N, 40P, 30K -Ino.	3.52	975	9,475	15,840	6,365
100N, 40P, 30K - Ino.	3.90	1,520	10,020	17,550	7,530
0N, 40P, 30K + Ino.	4.20	1,005	9,475	18,900	9,425
20N, 40P, 30K + Ino.	5.00	739	9,209	22,500	13,291

Source: Nguyen Huu Hiep et al. 2002

Table 3. Effect of N_2-fixing inoculants on yield of rice, maize and tea.

Trial No	Soil type and crops	Fertilizer	Yield (t ha^{-1})	% increase over control
1	Rice on fertile soil	Control: NPK (90:45:30) + 8t FYM (ground fertilizer, GF)	5.16	–
		80% GF + N_2-fixing inoculant	5.37	4.07
		GF + N_2-fixing inoculant	5.78	12.02
2	Rice on infertile soil	Control: NPK (90:45:30) + 8t FYM (GF)	**2.96**	–
		80% GF + N_2-fixing inoculant	3.44	16.22
		GF + N_2-fixing inoculant	3.54	19.59
3	Maize on fertile soil	Control:NPK (180:120:90) + 10t FYM (GF)	**4.14**	–
		80% GF + N_2-fixing inoculant	4.03	–2.66
		GF + N_2-fixing inoculant	4.53	9.40
4	Maize on infertile soil	Control: NPK: (180:120:90)+10t FYM	**2.95**	–
		80% GF + N_2-fixing inoculant	2.87	–2.72
		GF + N_2-fixing inoculant	3.25	10.20
5	Tea on Feralit soil	Control: NPK (120:60:60) (GF)	**14.29**	–
		70% GF + N_2-fixing inoculant	15.10	9.10
		GF + N_2-fixing inoculant	16.86	26.70

Table 4. The effect of N_2-fixing and P-solubilizing inoculants on the biocontrol of potato.

Treatments	% disease-affected plants			Crop yield (t ha^{-1})
	Bacterial wilt	Black root	Fungal root diseases	
Ground fertilizer (GF)	3	10	12	18.00
GF + 10%N	3	10	14	18.70
GF + *Klebsiella*	2	5	6	19.35
GF + *Pseudomonas*	2	5	6	19.38
GF + *Azotobacter*	1	5	6	19.60

Table 5. Effect of mixed inoculation of BNF and P-solubilizing microorganisms on rice production.

Treatment	Season	Experiment on small-scale		Profit (1000 VND)	Large-scale trial	
		Yield (t ha^{-1})	% increase		Yield (t ha^{-1})	% increase
Control	Spring	3.81	–	1,683.9	3.61	–
Inoculant		4.39	15.2	2,355.2	4.00	10.73
	$LSD_{0,05}$	0.324	–	–	–	–
Control	Autumn	4.28	–	4,348.4	4.15	–
Inoculant		4.95	15.7	4,716.6	4.67	12.52
	$LSD_{0,05}$	0.525	–	–	–	–

Table 6. Effect of mixed inoculation of BNF and P-solubilizing microorganisms on soybean production.

Treatment	Small-scale trial		Profit (1000VND)	Large-scale trial	
	Yield (t ha^{-1})	% increase		Yield (t ha^{-1})	% increase
Control	1.90	–	6,160.80	1.90	–
Inoculation	2.27	19.5	7,865.70	2.16	13.6
$LSD_{0,05}$	0.23	–		–	–

Table 7. Effect of *Frankia* on the growth of pine tree Mavi in the nursery.

Frankia preparation	Rate of infection (%)	Tree height (cm)	Tree weight (g tree^{-1})	
			Fresh	Dry
Control	0.0	87.45 *a*	15.70 *a*	5.16 *a*
Fr1	68.4	105.52 *d*	27.67 *c*	9.37 *c*
Fr2	55.2	93.90 *b*	18.98 *ab*	6.83 *abc*
Fr3	63.7	98.25 *c*	25.68 *bc*	8.46 *bc*

Means followed by the same letter in the same column were not significantly different (P>0.05)

Vietnam. The profits from *Rhizobium* inoculation on soybean are 3-6 million VND. Depending on the fertilizer status and nutrition content of soil, N_2-fixing inoculant can increase the yield of rice by 4.0-19.6%, of tea by 9.1-26.7% and of maize by 9.4-10.2%, with profits of about 15,000-481,000 VND ha^{-1} for rice, 0.1-0.28 million VND ha^{-1} for maize, and 1.46-4.63 million VND ha^{-1} for tea, respectively.

In addition, inoculants can improve plant resistance to some pathogen diseases. Results of field experiments with potato on fertile soil can be seen in Table 4, where there was significant decrease in some pathogen diseases after using N_2-fixing and P-solubilizing inoculant.

Mixed inoculant from N_2-fixing and P-solubilizing microorganisms is the new biopreparation being used. It has a positive effect on growth and

yield of different crops and consequently yields greater profits for farmers. Mixed inoculant can increase the yield of rice 15.2-15.7% and of soybean 16.3-19.5%.

BNF AND SOIL IMPROVEMENT IN SLOPING LAND

More than 50 ethnic groups inhabit the mountainous regions of Vietnam, and most of them rely on shifting cultivation with fallow period. The soil fertility depletes fast, and productivity is also reduced due to water and soil degradation. The living standards and the agricultural production of the highland farmers thus remain low and unstable. To identify, adapt, test and extend cropping systems that are both productive to meet farmers' interests, and environment-friendly to save and conserve resources base in the mountainous regions, the Vietnam Agricultural Science Institute (VASI) and CIRAD-France research soil improvement in severely degraded areas. Some species of legumes were grown and tested for soil improvement, fodder production and weed control (Table 8). The first results showed that cover crops could prevent soil erosion, and improve soil physical properties and soil fertility. Mulching with crop residues has many advantages such as prevention of soil erosion; soil fertility and structure improvement; increased soil moisture; neutralization of toxicity; stimulation of activities of soil microorganisms; preventing the spread of the airborne seed of most widespread upland weeds, and increased agricultural productivity (Table 9).

Table 8. Tested soil improvement legume and grass species.

No	Species name	Family	Possible use
1	*Aeschynomene histrix*	Leguminoseae	SI, AnF, Mu, WeC
2	*Calopogonium mucunoides*	Leguminoseae	SI, AnF, Mu, WeC
3	*Canavalia ensifotmis*	Leguminoseae	SI, AnF, Mu, WeC
4	*Chamaecrista rotundifolia*	Leguminoseae	SI, AnF, Mu, WeC
5	*Stylosanthes guianensis*	Leguminoseae	SI, AnF, Mu, WeC
6	*Mucuna mucunoides*	Leguminoseae	SI, AnF, Mu, WeC
7	*Pueraria phaseoloides*	Leguminoseae	SI, AnF, Mu, WeC
8	*Vigna umbellata*	Leguminoseae	SI, AnF, Mu, WeC
9	*Avena sativa*	Graminae	SI, AnF, Mu
10	*Brachiartia brizantha*	Graminae	SI, AnF, Mu, WeC
11	*B. humidicola*	Graminae	SI, AnF, Mu, WeC
12	*B.ruziziensis*	Graminae	SI, AnF, Mu, WeC
13	*Hordeum vulgare*	Graminae	SI, AnF, Mu
14	*Setaria italica*	Graminae	SI, AnF, Mu
15	*Sorgum bicolor*	Graminae	SI, AnF, Mu
16	*Paspalum atratum*	Graminae	SI, AnF, Mu
17	*Indigofera teysmanii*	Leguminoseae	SI, AnF, Mu, WeC
18	Hybrid *acacia*	Leguminoseae	SI, AnF, Mu

SI: Soil improvement, AnF: Animal feed, Mu: Mulch, WeC: Weed control

Table 9. Effect of soil mulching on crop yield in sloping lands.

Treatment	Yield (t ha-1)	% increase over control	Note
Rice:			
- Bare soil (control)	0.36	–	100 man days/ha weeding
- Mulched soil	0.80	22	20 man days/ha weeding
Maize:			
- Bare soil (control)	3.12	0	More weeding
- Mulched soil	4.01	28	Less weeding
Cassava:			
- Bare soil (control)	18.62	0	More weeding, more erosion
- Covered soil (*C. rotundifolia*)	24.92	34	Less weeding, more erosion
- Covered soil (*S. guianensis*)	26.88	44	Less weeding, more erosion

CONSTRAINTS OF BNF RESEARCH & DEVELOPMENT AND POSSIBLE SOLUTIONS

Although inoculant research and development began in Vietnam more than 20 years ago, production and application of inoculants are very limited. There is no factory producing sterile inoculant in the country – these are produced only by research organizations on a small scale. In general, peat is used as a carrier and sterilized by autoclave before inoculation with microbial biomass. Radiation sterilization technique is applied, but only for research purposes. These kinds of inoculants are of good quality, but due to lack of facilities, information and demonstration, they are used mainly in research projects. Thus, most of the inoculants applied in the field are nonsterile, produced by mixing microbial biomass and compost – these are of poor and unstable quality, so that their effect on plant growth and yield is not evident. In Vietnam, there are more than 10 companies producing 20 types of inoculants that are applied on large areas of rice, vegetable, maize, sugarcane, and fruit trees. Nonsterile inoculants contain both microbiomass and some nutritive elements like humus and mineral nitrogen, phosphorus and potassium, so the beneficial effect of microorganisms cannot be clearly determined.

BNF has positive effects on growth and yield of most agricultural crops and on soil fertility improvement. Although the BNF research program in Vietnam is well established, production and application is very limited. Reasons and possible solutions are:

- Extrapolation of research results from pilot tests to industrial production is incomplete. It is necessary to establish the technology developed on a production scale, rather than only at laboratory level.
- The poor quality of the inoculants has an adverse effect on farmers' perception of the technology. Thus, quality control is of great importance.

- On-farm demonstration and local training courses for extension workers and farmers can improve the knowledge and experience of the BNF user.
- International cooperation can help improve BNF research & development in Vietnam and other developing countries.

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Status of Biological Nitrogen Fixation Research in the Philippines

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ABSTRACT

This paper discusses the recent trends of BNF application in crop production in the Philippines. The priority programs of the PCARRD Medium Term and Development Plan (MTP) for CY 2000–2004 are on Natural Resources Management, which addresses schemes for sustainable development in general, and protection of environment and resource conservation and management through the utilization of organic agriculture (i.e. bioorganic fertilizers, soil management and agricultural biotechnology). The use of BNF as a component of integrated nutrient management should be considered for the development of a better agricultural system with an environment-friendly approach. The members of the National Resources Research and Development Network (NARRDN) of PCARRD implemented some research on BNF of certain agricultural crops and tree species. The research areas mainly focused on effectiveness of rhizobial inoculation on growth and yield, legume-*Rhizobium*-mycorhhizae interaction in crop production under acid soils, host protein variability and biochemical selection for improved nitrogen fixation, effect of cropping sequence on rhizobial population and commercialization of *Rhizobium* in mung bean production.

INTRODUCTION

The Philippines, one of the largest island groups in the world, lies at the western rim of the Pacific Ocean, north of the equator and about 1,000 kilometers from the Asian mainland. The total area of the country is about 30 million ha of which 12.9 million are cultivated.

The current thrust of the Philippine government is to agro-industrialize; however, it is still considered an agricultural country with more than 70% of an estimated 80 million Filipinos directly or indirectly involved in agriculture.

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Fifty one percent of the population is rural. The annual population growth rate of 2.8% has placed tremendous pressure on agricultural lands. Prime agricultural land is being converted to resettlement areas and put to industrial uses. And yet, with the decreasing area for agriculture comes the need to increase crop productivity to be able to support the increasing population. The current challenge lies in the application of sustainable farming systems that are environment friendly yet promote increased productivity.

The economic and environmental costs of the heavy use of chemical nitrogen fertilizers in agriculture have become a global concern and alternatives to nitrogen fertilizers must be urgently sought. (Bohlool et al. 1992). The country consumes about 1.92 million metric tons (Mt) of chemical fertilizer per year (Fertilizer & Pesticide Authority 2001). In the year 2000, the country imported around 1.26 million Mt (Table 1) of chemical fertilizer, of an estimated value of US$ 112 million. Aside from being a drain on the country's dollar reserve, the heavy use of chemical fertilizer also entails large expenses for the small-scale farmer.

A potential alternative to this heavy use of fertilizers is biological nitrogen fixation (BNF). Nitrogen-fixing systems offer an economically attractive and ecologically sound means of reducing external inputs and improving internal resources. This paper discusses the status of BNF research in the Philippines.

Table 1. Fertilizer: Total imports by grade, 1995-2000 ('000 Mt).

Type (N-P-K)	1995	1996	1997	1998	1999	2000[P]
Urea	651.89	660.07	640.51	550.66	682.00	577.71
15.5-0-0	1.57			0.62	0.38	0.41
16-0-0					6.76	
21-0-0	282.08	166.31	240.90	162.77	217.45	294.26
25-0-0	35.92	15.00	27.59	0.32	3.15	3.45
27-0-0	0.20		0.30	6.64		
0-18-0				5.53		
11-52-0						
16-20-0	6.26			2.20		43.83
18-46-0	78.50	143.44	79.72	33.24	110.00	148.19
20-20-0					0.061	
14-14-14						12.00
16-16-16			6.24		5.00	4.00
0-0-52	0.17					
0-0-60	179.05	205.54	226.29	35.93	186.16	172.34
$MgSO_4$				0.10		
$FeSO_4$				0.60		
$ZnSO_4$	1.92	7.48	1.99	0.95	1.65	1.10
KNO_3		4.01	5.98	2.07	9.74	2.56
Kieserite			1.91			
Total	1,237.58	1,202.16	1,246.10	786.66	1,222.35	1,259.86

Source: Fertilizer and Pesticide Authority, Department of Agriculture, 2001.

THE PCARRD MEDIUM TERM AND DEVELOPMENT PLAN

The Philippine Council for Agriculture, Forestry and Natural Resources Research and Development (PCARRD 2000) 2000-2004 Medium Term Plan (MTP) outlines the Council's programs and projects toward the realization of its vision as a "responsible and creative Science and Technology (S & T) leader and effective institutional partner for productive and scientific research that will sustain and enable the country's agriculture, forestry and natural resources sectors to be competitive, environmentally sound and socially relevant while ensuring farmers' and clients' income and livelihood."

Thus PCARRD, as a sectoral planning council of the Department of Science and Technology (DOST), will ensure that its Research and Development (R & D) policies, programs and activities will enable the R & D institutions to produce scientific research that will benefit the people and the industries dependent on agriculture and forestry for their livelihood and income opportunities with due regard to environmental sustainability.

To realize its vision and mission, PCARRD implements the following four major strategies:

- Focusing R & D on high-end sciences, technologies and management systems for sustained growth;
- Enhancing technology management, promotion and commercialization;
- Improving R & D capability and governance; and
- Formulating and advocating policies for S & T development.

As part of the first strategy, there is a program on environment and natural resources management. The priority programs on natural resources management address sustainable development in general, as well as environmental protection, resource management and conservation, and promotion of organic agriculture.

Also part of the first strategy, PCARRD has a banner program on agriculture and forestry priorities in biotechnology. One of the priorities of this program is the use of applied genomics on economically important soil microorganisms to ensure continuous maintenance of soil organic matter and fertility and BNF.

PCARRD has established the National Agriculture and Resources Research and Development Network (NARRDN) to implement its R & D programs. The network is composed of four national multi-commodity R & D centers, eight national single-commodity R & D centers, eight regional R & D centers, and various cooperating stations and specialized agencies. One of the national multi-commodity research centers of NARRDN is the University of the Philippines, Los Baños (UPLB) where two units have

conducted research on BNF for some agricultural crops and tree species. These are the National Institutes of Biotechnology and Applied Microbiology (BIOTECH) and the College of Forestry.

BIOLOGICAL NITROGEN FIXATION RESEARCH

Institutions working on soil fertility management, reforestation and biotechnology have conducted research on BNF. The National Institutes of Biotechnology and Applied Microbiology of UPLB has developed a commercial product known as Nitro Plus. Nitro Plus contains effective rhizobia specific for legumes such as peanut, mung bean, cowpea, pole sitao and soybean (dela Cruz 1993). Effective inoculation of Nitro Plus into plants causes nodule formation.

Nitro Plus can partly replace application of nitrogen fertilizers. The inoculant is economical since it is cheaper than nitrogen fertilizers and practical too since it is simply applied by coating the seeds before they are sown.

Another commercially available product developed by BIOTECH is the BIO-N, a solid inoculant in powder form. This product is being produced from *Azospirillum*, a bacterium isolated from the roots of the local grass, talahib (*Saccharum spontaneum* L.). When inoculated, the bacteria rapidly colonize the roots, and fix atmospheric N_2. Several strains have been isolated and some of these were found effective in promoting the growth of rice, corn, and sweet potato. BIO-N comes in a handy 200-gram packet, and about 5-6 packets are recommended per hectare of rice or corn.

One BNF research area focused on the effectiveness of rhizobial inoculation on growth and yield of soybeans (Asanuma et al. in Palis 1999), mung bean (Paterno et al. 1990), cowpea, peanut and snap beans (Noguchi et al. in Palis 1994) in a field where the soil order was Ultisol. Ultisols have pH of 6.0 or less and almost all nutrients including nitrogen are deficient. Noguchi's results showed that inoculation with rhizobia alone increased shoot dry weight of peanut by as much as 30% at 63 days after planting. The acetylene reduction activity of the rhizobia-inoculated plants also increased. Dual inoculation with rhizobia and mycorrhiza however, gave a higher acetylene reduction activity.

In one of the studies conducted by Paterno et al. (1999), evaluation of different legume-*Rhizobium*-mycorrhizal (VAM) combinations showed the beneficial effect of mycorrhizal application on dry matter yield and phosphorus uptake of mung bean grown in acid soil at both 0 and 60 kg phosphorus application. Acid-tolerant accessions were also found to perform better than the intolerant entries when tested in terms of dry matter yield and phosphorus uptake.

To promote the inoculation technology, farmers' utilization and improvement of legume inoculation technology was studied (Paterno et al. 1997). Peanut and mung bean were inoculated in five regions with farmers as cooperators. In the on-farm trials, granular inoculation gave the highest seed yield of peanut.

One study aimed to utilize four nodule enzymes: glutamine synthetase (GS), glutamate synthase (GOGAT), glutamate dehydrogenase (GDH) and phosphoenolpyruvate carboxylase (PEPC) as additional selection parameters for improved N_2 fixation (Hautea et al. 1994) based on the nitrogenase activity measured by acetylene reduction assay (ARA). Biochemical and genetic studies were conducted on these four enzymes in mung bean and cowpea. In mung bean, results indicated that nodule GS and PEPC activities may have an effect on the N_2-fixing ability of the plant but the effects of these factors interact with other factors which greatly influence the N_2 fixation potential of a plant at a given time. They concluded that a more thorough investigation using greater number of genotypes is needed to confirm the results before the use of nodule enzymes as selection parameters in breeding for enhanced N_2 fixation could be promoted. Besides, the precise role of the nodule enzymes in N_2 fixation remains to be determined.

Paterno et al. (in PCARRD Legumes R & D Status and Directions 2001) conducted a study on the behavior of rhizobia in rice-based cropping system and found that:

- rhizobial count decreased with prolonged flooding but increased when field was drained at rice harvest;
- nodule occupancy by the inoculum strains decreased with cropping from 96% in the first rice crop to 40% in the third crop;
- population of rhizobia generally decreased during rice culture. It took three soybean seasons to establish a large soil population of *Bradyrhizobium japonicum*;
- cowpea rhizobial population under continuous lowland rice crops slightly increases the number of cowpea rhizobia in the soil to about 103 rhizobial cells g^{-1} of soil.

Besides crop production, BNF studies in reforestation were also done. Nitrogen-fixing organisms in species that thrive well in reforestation areas help the species survive the prevailing conditions of the site. N_2-fixing bacteria and *actinomycetes* form symbiotic association with the roots of plants to form root nodules. BNF on land was estimated to be 140 t yr^{-1} and this has been taken advantage of in upland farming or agroforestry practices because of the value of nodulated trees in soil amelioration (Garcia 1991). BNF in agroforestry is utilized in intercropping legume with nonlegume and green manuring as practiced in alley cropping.

Survey, isolation and screening for the most effective rhizobia for multipurpose tree species have been conducted at the College of Forestry and BIOTECH in UPLB since 1979. *Rhizobium* inoculants for legume trees such as *Pterocarpus indicus, Acacia mangium, A. auriculaeformis, Samanea saman, Paraserianthes falcataria, A. procera, Sesbania grandiflora* and *Gliricidia sepium* are already available. Studies suggest that these species could be enhanced through phosphorus fertilization and combined inoculation with endomycorrhizae (Lapitan and Garcia 1993). Some studies show that a minimal input of combined N (20-50 kg N ha^{-1}) helped initial growth and consequent nodulation of *Acacia, Albizia* and *Leucaena*. Other studies indicate that slight adjustment of pH from 4.0 to 6.0 improved nodulation and growth of certain trees.

There were also reports that in *A. mangium*, inoculation independently improved height, shoot biomass, nodule weight, and nitrogen content and uptake (Cali in Lapitan and Garcia 1993). Inoculating *A. auriculaeformis* with UPLB *Rhizobium* isolates Aa2 and Aa3 significantly improved seedling height, dry matter yield and nodulation. It produced appreciable increases in nitrogen, phosphorus, potassium, calcium and magnesium uptake by plants (Garma in Lapitan and Garcia 1993).

Four projects were conducted in nutrient management (PCARRD, Agricultural Ecosystems R & D Status and Directions 2001). These dealt mainly with improvement of efficiency and environmental impact of nitrogen fertilizers through their effective management, nutrient management in rainfed cropping systems and carbon dynamics, nutrient cycling, and the sustainability of cropping and pasture systems.

FUTURE PROSPECTS OF BNF RESEARCH

The accomplishments in BNF research so far have to some extent contributed to the effort to increase crop productivity. However, in the past, researchers focused only on some aspects such as microbial inoculation, and a few crop species and cropping systems. Not much attention has been given to the integrated management of soil nutrients in the various agroecological zones and the socioeconomic impacts of technologies. Much remains to be done yet and BNF holds great promise. The advent of biotechnology can also spur the use of molecular genetics to enhance BNF but this needs upgrading of the human resource capability in the country.

Therefore, to address the global concern on food security and to achieve progress in alleviating poverty, there is an urgent need for all stakeholders to strengthen their partnership in the development of a practical program on BNF.

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Effect of Fertilizers on Nodulation and Growth of *Samanea saman* Merr in Presence of Arbuscular Mycorrhizal Fungi at Nursery Level

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ABSTRACT

The influence of different fertilizers on the nodulation and growth of *Samanea saman* Merr (Syn. *Albizia saman*) in presence of arbuscular mycorrhizal fungi (AMF) was studied under nursery conditions. Before sowing of seeds, different combinations of N, P, K fertilizers were incorporated with the nutrient deficient natural forest soils that was again amended with 10% cow dung and 10% AMF soil inoculum. Nodulation status (nodule number, shape, fresh weight, dry weight, distribution and color) in roots and plant growth parameters (shoot height, root length, collar diameter, fresh and dry weights) of the plants were recorded 45 days after seed germination. In the presence of AMF, nodulation status and growth of the plant differed widely in the soils amended with fertilizers in comparison to control. The highest number of nodule was recorded with the lowest dose of NPK (0.25 g kg^{-1} soil) and highest fresh and dry nodule weight was found with lower dose of NPK (0.5 g kg^{-1} soil) treated plot. Nodule shape and color also varied widely in different treatments. In case of plant growth parameters, shoot height and collar diameter did not differ significantly ($p<0.05$) but root length, fresh and dry weight of plants differed significantly in different combination of fertilizers. From the study, it can be concluded that for optimum growth and nodule formation of *S. saman* in degraded sites, 1/4 g NPK (1:2:1) fertilizer kg^{-1} soil mixture (degraded soil+10% AM inoculum + 10% cow dung) is recommended at nursery level.

INTRODUCTION

Samanea saman Merr (Syn. *Albizia saman*) is a fast growing N_2-fixing tree (NFT) with spreading crown. The tree is widely distributed in the tropical forests of

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Asia. It grows well in well-drained neutral to slightly acidic soils (Zabala 1990). The fruit is a pod with sweet pulp that is eaten by wild animals. In Bangladesh, the tree is widely planted in degraded soils, roadsides, homesteads, parks and marginal lands.

Nitrogen-fixing trees are ideal for afforesting degraded soils (MacDickens 1994) because of their ability to establish and thrive in nitrogen deficient soils. If the fertility of degraded soil is to be maintained, it must be replenished either by fresh supply of fertilizers or by plants that regularly fix atmospheric N_2. Although inorganic fertilizers are expensive to use over vast plantation areas, they greatly influence growth and formation of nodules (Pankaj et al. 1998). Bio-fertilizers such as arbuscular mycorrhizal (AM) fungi and *Rhizobium* do not have adverse effects on soil systems. On the contrary, they help boost the microbial population present in the soil, which in turn makes insoluble nutrients available for plant growth (Verma et al. 1996)

Much research has been done on the effect of fertilizers on growth and nodulation in different legumes (Ginwal et al. 1995, Perez et al. 1996, Singh et al. 1995) in the presence of biofertilizers. But there is little data in the case of *S. saman* (Datta and Das 1997), especially in soil conditions in Bangladesh. This study is an attempt to record the growth and nodulation status of *S. saman* in presence of AM fungi in natural degraded soils amended with different fertilizers, without artificial inoculation.

MATERIALS AND METHODS

Site Selection and Plot Preparation

The study was conducted at the nursery of the Institute of Forestry and Environmental Sciences, University of Chittagong (IFESCU), Bangladesh. Natural degraded soils were collected from hilly sites in Chittagong University Campus. The collected soils were sieved (<3mm sieve) to get a uniform soil size. The degraded soil contained total N 0.23 %, P 2.0 ppm, and K 0.18 meq 100^{-1} g (Rahman and Mridha, in press). Cow dung was collected locally and deposited in the IFESCU nursery to decompose for about two months. Then the decomposed cow dung was also sieved (<3mm sieve). The sieved soil and cow dung were mixed uniformly in the ratio of soil:cow dung = 9:1. Thirty-six plots containing twelve treatments were arranged randomly in the nursery bed in three lines. Each plot size was 70cm X 60cm X 10 cm. Arbuscular mycorrhizal soil inoculum produced in the Mycorrhizae Laboratory of Chittagong University was thoroughly mixed (10% – 1 kg per 9 kg of soil) with the soil. The AM soil inoculum contained fungal spores plus mycorrhizal roots @ 250 per 100g soil. Different combinations of urea (N 46%), Triple Super Phosphate (P_2O_5 48%) and muriate of potash (K 50%) fertilizers were applied to the soils. Seed sowing was done three days after mixing the fertilizers in the plot.

Seed collection and experimental design

Fruits were collected from the plus (healthy selected) *S. saman* trees of the University campus. They were dried in the sun, and seeds were then extracted. Uniform seeds were selected for the experiment. The seeds were sown in the nursery bed in April 2002 and allowed to grow up to 45 days. A randomized complete block design with three replicates was used in the study. One thousand and eighty seeds were sown in twelve different treatments. Three replications of each treatment comprised ninety seeds. The seeds were sown at equal depth (2 cm) in random plots. The treatments used in the experiment were as follows:

Group	Treatment
T1	Only soil (control)
T2	Soil + cow dung
T3	Soil + cow dung + AM inoculum
T4	Soil + cow dung + AM inoculum + NPK (N: P: K = 1:2:1) @ 1 g kg^{-1} soil
T5	Soil + cow dung + AM inoculum + NPK (N: P: K = 1:2:1) @0.5 g kg^{-1} soil
T6	Soil + cow dung + AM inoculum + NPK (N: P: K = 1:2:1) @0.25 g kg^{-1} soil
T7	Soil + cow dung + AM inoculum + N @1 g kg^{-1} soil
T8	Soil + cow dung + AM inoculum + P @1 g kg^{-1} soil
T9	Soil + cow dung + AM inoculum + K @1 g kg^{-1} soil
T10	Soil + cow dung + AM inoculum + NP (N: P = 1:1) @1 g kg^{-1} soil
T11	Soil + cow dung + AM inoculum + NK (N: K = 1:1) @1 g kg^{-1} soil
T12	Soil + cow dung + AM inoculum + PK (P: K = 1:1) @1 g kg^{-1} soil

Assessment of Growth and Nodule Parameters

The seedlings were harvested 45 days after germination. Ten seedlings from each plot were randomly selected and carefully collected with the entire roots intact. Five of these were used to assess nodule parameters (nodule number, nodule fresh and dry weight, color, shape, size, and distribution) and the other five to estimate the growth parameters. After taking the shoot height, root length, collar diameter, shoot and root fresh weight, the seedlings were oven dried for dry weight at 70°C for 48 hours until a constant weight was obtained. The data were averaged for nodulation status and growth parameters in each replication and were analyzed statistically using Duncan's Multiple Range Test (DMRT).

RESULTS

The nodule number, fresh and dry weight, color, shape and distribution of nodules in different treatments of *S. saman* have been shown in Table 1. All the treatments showed nodule formation but these varied widely.

Significantly ($p<0.05$) high nodule number (15.00) was found in T6 followed by T5, T4, and the lowest (0.66) in T7 treatment. The highest nodule fresh and dry weight was recorded in T5 followed by T8, T6, T11, and the lowest in T1 treatment. Most of the nodules were brown (T2, T3, T4, T5, T6, T8, T10, T11), whitish brown (T1) or reddish brown (T12) while T7 showed whitish and T9 showed pinkish color. Nodule shape in different treatments varied from globose to semi-globose (T3, T4, T5, T6, T10 and T11), elongate to elongate with clusters (T1, T2), circular (T8, T9, T12) and kidney shaped (T7). All the nodules were distributed in the secondary roots. All mycorrhiza-incorporated treatments (except in N-amended soil) showed higher nodule status than the control treatment.

Table 1. Nodule number, nodule fresh and dry weight, color, shape and distribution in different treatments of 45-day old *Samanea saman* seedlings.

Treatments	Nodule number	Nodule weight (g)		Nodule colour	Nodule shape	Nodule distribution
		Fresh	Dry			
T1	1.00 e*	.014 c	.007c	Brown to white	Elongate	Secondary roots
T2	4.00 cde	.015 c	.008c	Brown	Elongate with cluster	Secondary roots
T3	3.33 de	.020 bc	.012bc	Brown	Semi globose	Secondary roots
T4	13.33abc	.035 bc	.028bc	Brown	Globose	Secondary roots
T5	14.33 ab	.094 a	.080a	Dark brown	Semi globose	Secondary roots
T6	15.00 a	.044 abc	.031abc	Brown	Semiglobose	Secondary roots
T7	0.66 e	.047 bc	.033bc	Whitish	Kidney shaped	Secondary roots
T8	12.66 abcd	.067 ab	.054ab	Brown	Circular	Secondary roots
T9	7.00 abcde	.043 bc	.030bc	Pinkish	Circular	Secondary roots
T10	8.33 abcde	.035 bc	.025bc	Dark brown	Semi globose	Secondary roots
T11	5.30 bcde	.043 bc	.028bc	Brown	Globose	Secondary roots
T12	12.30 abcd	.042 bc	.027bc	Reddish to brown	Circular	Secondary roots

Note: *Means followed by the same letter (s) are not significantly different ($p<0.05$) according to Duncan's Multiple Range Test (DMRT)

The growth parameters like shoot height, root length, collar diameter, and root-shoot fresh and dry weights varied in different treatments (Table 2). The highest shoot height was recorded in T11 and the lowest in T1, but they did not vary significantly ($p<0.05$). Similarly, the highest significant root length was found in T3 and the lowest in T5 treatment. Collar diameter did not vary significantly. Shoot fresh and dry weight was significantly highest in T6 and the lowest in T1 and T3 treatments. The highest significant root fresh and dry weight was found in T12 and the lowest in T8 treatment.

Table 2. Shoot height, root length, collar diaameter, shoot fresh and dry weight, root fresh and dry weight of 45 days old *Samanea saman* seedlings in different treatments.

Treatments	Shoot height(cm)	Root length(cm)	Collar diameter (mm)	Shoot weight (g)		Root weight (g)	
				Fresh	Dry	Fresh	Dry
T1	8.33 a *	6.83 cd	1.10 a	.40 g	.21 g	.58 c	.05 c
T2	10.16 a	8.83 bcd	1.46a	.46 f	.22 f	.12 bc	.06 bc
T3	10.00 a	11.50 a	1.06a	.38 g	.15 g	.08 c	.04 c
T4	10.50 a	10.43 ab	1.40a	.76 c	.35 c	.07 c	.03 c
T5	10.23 a	5.80 d	1.26a	.66 d	.31 d	.07 c	.03 c
T6	8.93 a	7.40 cd	1.23a	1.03 a	.51 a	.13 b	.07 b
T7	8.33 a	6.16 cd	1.16a	.63 d	.32 d	.11 bc	.05 bc
T8	8.90 a	7.33 cd	1.13a	.80 c	.38 c	.06 d	.04 c
T9	9.83 a	6.40 cd	1.16a	.95 b	.42 b	.12 bc	.06 bc
T10	10.10 a	8.80 bc	1.33a	.58 e	.22 e	.14 b	.07 b
T11	11.83 a	6.10 cd	1.43a	.55 e	.24 e	.15 b	.08 b
T12	10.76 a	6.16 cd	1.33a	.54 e	.24 e	.72 a	.31 a

Note: *Means followed by the same letter (s) are not significantly different ($p<0.05$) according to Duncan's Multiple Range Test (DMRT).

DISCUSSION

The findings of the present study indicate that nodulation status (i.e. number of nodules per plant, shape, color and fresh and dry weight of nodule) recorded from different fertilizer treatments in the roots of *S. saman* varied in the presence of AM fungi. The data is in agreement with the work of Datta and Das (1997), who reported a positive relationship between nodule dry weight and biomass, whereas our work shows no direct relationship between nodule wt and biomass. Increased fertilizer treatment resulted in increased growth and dry matter production with reduced nodule numbers in the present study (with few exceptions). The lower rates of NPK fertilizer (1/4 g kg^{-1} soil) is more effective than higher rates (1g kg^{-1} soil) in nodule formation and number of nodules per plant, while K having little effect. Ravichandran and Balasubramanian (1997) recorded similar results with *Casuarina* seedlings and Gupta et al. (1998) with *Leucaena leucocephala* to various levels and sources of fertilizers. Maschio et al. (1997) found higher rate of mycorrhizal formation increases nodule dry weight (0.18 g) and overall development, despite a very poor root system in some legumes including *Tipuana tipu*.

Perez et al. (1996) studied the effect of fertilization on nodulation and root growth of *Acacia mangium* in the nursery and found that nodule weight was highest with low dose of NPK (0.0125 g/nodule), and least with NK alone (0.0015 g/nodule). In this study, nodule weight was greater (0.094 g/nodule) with the NPK lower dose (0.5 g NPK kg^{-1} soil), and lesser with degraded soil alone (0.014 g/nodule). Masuka (1995) found that nitrogen consistently had a negative effect on both nodule number and weight, and

root weight. Razz et al. (1995) studied the effect of N and P fertilizer on nodulation and found that N affected the size of nodules while P increased nodule number. Paulino et al. (1995) found in *Leucaena leucocephala* that shoot dry weight and nodule numbers and weight increased with increasing K fertilizer dosage, while N and P had less effect. Shaukat (1994), in his study the effect of nitrogen and phosphorus fertilizers on root growth and nodulation, noticed that frequent application of N resulted in increased root weight and reduction in the number of nodules. Phosphorus fertilizing increased root weight, but phosphorus alone had no effect on the number of nodules. In the present study, nodule formation and growth parameters were seriously affected in N (1 g kg^{-1} soil) amended soil, which is comparable to observations recorded by others.

CONCLUSION

The present study shows that 0.25 g NPK (1:2:1) fertilizer per kg soil mixture (degraded soil + 10% AM inoculum + 10% cow dung), if applied at nursery level, facilitates optimum growth and nodule formation in *S. saman*, and helps obtain healthy seedlings that can be easily established in degraded sites.

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IV
The CP Pre-Proposal

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Biological Nitrogen Fixation for Increased Crop Productivity, Enhanced Human Health and Sustained Soil Fertility: A Challenge Program Pre-Proposal*

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INTRODUCTION

Nitrogen is, with water, the greatest global constraint to agricultural productivity. Sustainable cropping systems throughout history have relied on combining cereals with N_2-fixing legumes. However, over the last fifty years, cereals have dominated global agriculture, while legume cultivation areas and productivity have stagnated or even declined. Global agriculture is now at a crossroads as a consequence of climatic changes, increased population pressure and detrimental impacts on the environment. Legumes should play a pivotal role in developing new strategic approaches to ensure sustainable increase in agricultural productivity, without harming the environment.

The Challenge Program on legumes and biological nitrogen fixation (BNF) is positioned in an *'innovation systems'* analytical framework, which analyzes the relationship between agricultural research innovations, market policies and development. To meet development needs and opportunities, and to ensure the economic and ecological sustainability of agriculture, approaches to BNF-legumes systems must be dynamic and innovative. One of these approaches is a multidimensional integrated approach to soil fertility management. Another is the exploitation of advances in plant genomics and bioinformatics.

*Based on outputs and contribution of all participants of the International Workshop (Montpellier 10-14 June, 2002)

Mission Statement

A major opportunity exists in many agroecosystems of the world to intensify the role of N_2-fixing legume crops. This increased focus on agro-diversification will significantly contribute to the livelihoods and health of rural households while substantially benefiting overall system productivity and sustainability. In the short term, this Challenge Program intends to identify and characterize these niches for targeted introduction of N_2-fixing legumes within agroecosystems around the world, and particularly resource-poor systems in Africa, South Asia and Latin America. This will form a framework for long-term holistic approaches to legume intensification based on the synergistic integration of research – in natural resource management, socioeconomic analysis, genomics and bioinformatics – to farmers' fields and to the market place. It is essential that strategies be developed that enable stakeholders, and more especially, the clients – the farmers and the marketplace – to participate in the whole R&D process, rather than to merely assume the role of the object of the exercise. The ultimate integration of legumes and microsymbionts into crop/ livestock systems is seen as a key step towards a fully integrated soil fertility management approach that will enhance crop production and contribute to the overall health of ecosystems and the people who live in them.

INTRODUCTION TO THE PRE-PROPOSAL

This pre-proposal originates from consultations with scientists and agricultural development specialists from CGIAR centers (in particular ICRISAT, CIAT, IITA and ICARDA), plus AVRDC, NARS, regional and sub-regional forums (APAARI, CORAF, ASARECA, FARA), advanced research institutes and international organizations (FAO, IAEA). A specific stakeholder consultation workshop was organized to support the preparation of the pre-proposal, at Montpellier, France, 10–14 June 2002. One of the outcomes of these consultations was that symbiotic nitrogen fixation (SNF) is a highly important element of any strategy to reverse the degradation of cultivated lands in many parts of the world. Particularly for the poorest farmers who cannot afford inorganic fertilizers for staple crops, BNF is essential to improve food security, soil fertility and livelihood.

Important opportunities exist in the short, medium and long term for enhancing the global role of legumes in cropping systems.

In the short term, it is urgent to collate the existing knowledge about BNF and legumes from public and private agencies. To enhance the uptake of N_2-fixing legumes by farmers, the dual demands of improved food production and enhanced system sustainability must be met.

The medium term objective is to optimize BNF by developing improved, low-phosphorus efficient, drought-adapted and disease- and pest-resistant

genotypes. Promoting the widespread adoption of these genotypes will lead to an increase in inputs from N_2 fixation in marginal environments.

In the long term, the prospects of biotechnology, particularly genomics and bioinformatics, could lead to dramatic improvements in N_2 fixation and other important adaptive traits in a wider range of legumes offering much greater choice for farmers across a wide range of environments.

BNF is a key element of any integrated soil fertility management strategy. The development and application of BNF techniques must be undertaken with continuous input from farmers, development agencies and policymakers.

BACKGROUND AND RATIONALE

The Challenge: A Green Revolution in Marginal Cropping Systems

During the 20th century, cereal yields increased to remarkable levels in many areas. These increases were largely facilitated by the introduction of genetically improved cultivars combined with the intensive use of agrochemicals. Meanwhile, however, productivity of low-input agriculture on marginal lands has stagnated or even declined. A critical challenge is that world food security cannot be ensured without dramatic increases in crop yields to cope with increasing population pressure, and this must be achieved while arresting or reversing degradation of agroecosystems.

Throughout history, civilization has depended on cropping systems that combine a cereal with a legume. But in recent history the system has become skewed – cereal production has dramatically increased whilst that of legumes has not. This imbalance is unsustainable and must be rationalized as efficiently as possible.

The improvements in cereal grain production that culminated in the Green Revolution resulted from the selection of N-responsive crop varieties with large reproductive structures to accommodate more grain and increased N through provision of fertilizer. Nitrogen is the soil nutrient needed in greatest quantity by crops. Although abundantly available in the air (78% of the atmosphere), plants cannot directly utilize gaseous nitrogen. BNF occurs naturally in legumes by forming a symbiotic association with N_2-fixing bacteria. Between 1950 and 1990, per capita fertilizer use quintupled and total fertilizer use increased ten-fold. At the same time, crop land per capita *decreased* by nearly 50%. The world currently spends more than $20 billion annually for fertilizer nitrogen. The high costs of N fertilizer production and dependence on non-renewable energy sources, combined with the potential economic and environmental benefits of BNF systems, have prompted substantial research investments in BNF-legume technologies.

The direct and complex causal relationships between loss of soil fertility, environmental degradation and poverty are firmly established. Traditional fallow systems in Africa have declined, resulting in significant losses of soil fertility, and mineral fertilizer use is limited for economic and infrastructural reasons. For most resource-poor farmers in developing countries, the gap between the inputs from external sources and the constant mining of soil nutrients is growing ever larger.

Estimates of soil nutrient losses in Sub-Saharan Africa, Asia and Latin America suggest a net removal of between 20 and 70 kg ha^{-1} of N from agricultural land each year, and these losses are likely to increase. Depletion of soil fertility is the most fundamental cause of low per capita food production in sub-Saharan Africa. Replacing the net depletion of soil nutrients in Sub-Saharan Africa alone would cost at least $4 billion *annually*.

In a global scenario of reaching a nitrogen resource plateau and rising concerns over possible environmental effects of chemical fertilizers, as well as their cost for small-scale farmers in developing countries, it is essential to expand the use of the BNF technologies that offer the greatest environmental and economic benefits for each specific agroecosystem.

In addition to their N_2-fixing capacity, legumes are extremely important in human and animal diets. Globally, they supply 33% of human protein. Legumes like groundnuts and soybeans are important sources of oil. Other legumes are a valuable source of unique phytochemicals that promote cardiac health. Some provide essential minerals like iron and zinc. Legume intensification within the farming systems also contributes to inter-seasonal food security, reduced stress, lower migration rates, and enhanced nutrition status of women and children. In short, legumes significantly increase household health standards.

BNF Between Past Failures and Future Prospects

Is a new 'Clean and Green Revolution' based on integrated management of soil nutrients and biological alternatives to chemical fertilizers possible today? Despite previous failures caused largely by single-point intervention approaches, optimism is now widespread that holistic approaches fueled by modern technologies can bring about substantial improvements.

BNF is, of course, already making a significant contribution as regards the total N fixed globally (about 90 million tons per year), but the need for its improvement and widespread application in agriculture has never been more urgent than it is today, especially for the improvement of the world's most vulnerable livelihoods and cropping systems.

Past research on BNF in most agricultural research institutes focused largely on specific aspects such as microbial strain selection and inoculation. Moreover, the focus was confined to a limited number of crop species and

cropping systems. Very little attention was given to the integrated management of soil nutrients or the socioeconomic impacts of technologies.

For example, adaptation studies of symbiotic N_2-fixing legumes to environmental constraints have been scarce and insufficient. The use of BNF technologies has often been discouraged by national policies that reduce the economic competitiveness of BNF, such as subsidization of chemical fertilizers. Little has been done to assist NARS in developing appropriate strategies and understanding socioeconomic issues for BNF technology adoption, or to identify robust marketing chains for legume grains. As a result, implementation of a holistic approach for sustainable crop productivity and soil fertility improvement has been extremely difficult. Consequently, the average quantities of N_2 fixed by legumes annually are lower than 30-50 kg ha^{-1}. Taking into account the small areas sown to legumes, inputs are as low as 5 kg ha^{-1} in many African farming systems. These inputs could potentially be at least 4-5 times higher. Different environmental factors, such as phosphorus deficiency, drought, acidity or alkalinity of soil and excessive applications of nitrogen fertilizer affect the in situ N_2-fixing activity, and limit the optimal contribution of BNF in cropping systems.

For many poor farmers, BNF is an essential, cost-effective alternative or complementary solution to industrially manufactured N fertilizers, particularly for staple crops. Many grain legumes are major sources of protein for the subsistence of poorest farmer households. When legume production exceeds household requirements, it can be readily traded to generate income, making significant and direct contributions to livelihoods. In addition to the considerable economic interest and positive impact on human health, BNF technologies have the potential to generate global environmental benefits by reducing greenhouse gas emissions and water pollution from inorganic N fertilizers. Research on BNF, particularly molecular genetic understanding of *Rhizobium*-legume symbiosis, has recently made significant progress, opening new possibilities to design strategies aimed at enhancing N_2-fixing capacity and legume productivity.

Synergies with the Genomics Revolution

Genomics is revolutionizing research and commerce in the life sciences, and offers to support the development of a new paradigm in the genetic improvement of legumes that will substantially augment the impact of BNF technologies. Following the whole genome sequencing of *Arabidopsis*, it became clear that a number of model hubs would be required throughout the plant kingdom to stimulate rapid impacts across important crop groups. Based on the remarkable synteny between grass genomes, the sequencing of the rice genome is expected to stimulate a cascade of scientific impacts on a wide variety of cereal crops.

Among the legume species, the most economically important crop, soybean, has failed to take a similar lead. Instead, the USA, the EU and Japan have committed tens of millions of dollars for the large-scale genome sequencing of two new model systems: *Medicago truncatula* and *Lotus japonicus*. These two species have quickly become models for structural and functional legume genomics. The species were initially chosen for their importance to research on SNF. However, they have quickly become models for the analysis of a wide variety of agronomically important traits.

For example, a systems biology approach is being undertaken within the EU-funded LOTUS program, applying functional genomics to answer ecophysiological questions. This will dramatically increase our understanding of how legumes respond to abiotic stress, which will then provide the basis for novel strategies for stress tolerance breeding. This research will generate a global understanding of the expression, regulation, dynamics and evolution of an array of agronomic characters, including those related to symbiotic associations.

By virtue of genetic synteny, this wealth of knowledge from the model legumes will leap-frog progress to the lesser studied legumes so important to agricultural systems, notably those in marginal areas. A critically important issue here is that developing countries be given the opportunity to conduct their own biotechnological research to resolve their own problems in their own way. The CGIAR centers' role is to ensure that the outputs from the genomics revolution are translated into appropriate applications and significant impacts.

PROGRAM OBJECTIVES

The main goal of the Challenge Program on BNF (CP-BNF) is to enhance legume cultivation, productivity and N_2-fixing rhizobial symbiosis for food production and soil fertility. This will help mitigate, through a participatory process, the downward spiral of soil fertility decline, food insecurity, malnutrition and poverty. The intention of the CP-BNF is to double global mean yields of major grain legumes from 0.7 t ha^{-1} to 1.4 t ha^{-1} within a decade and have a major impact on improving the livelihoods of the rural poor.

Key intermediate objectives are:

1. Development of integrated soil fertility management with options aiming at optimal N_2 fixation by the legumes, in combination with strategic applications of mineral fertilizers and other rhizospheric activities (e.g., P solubilization) to optimize the overall effects of the legume on the cropping system.

2. Cooperation with advanced genomics research labs to effectively translate the massive legume genomics and rhizobiology knowledge being generated in model species and major legume commodities for effective application in appropriate target areas for enhanced legume productivity.
3. Identification and development of legume germplasm that integrates SNF efficiency with stress adaptation in multipurpose grain, forage and tree legumes, and incorporation of germplasm with enhanced potential in rotational and mixed cropping systems.
4. Technology transfer to NARS implementing modern approaches to plant breeding, thereby facilitating widespread genetic improvement of legumes adapted to specific local conditions, and dissemination of BNF technologies for better adoption of INM options.
5. Development of efficient seed and rhizobial inoculum delivery systems and quality control, and creation of efficient marketing strategies and adequate institutional environment, to provide incentives to farmers for adopting N_2-fixing legumes.

STRATEGY OF IMPLEMENTATION

CP-BNF will be implemented through five interconnecting components (Fig. 1) in five major target ecoregions:

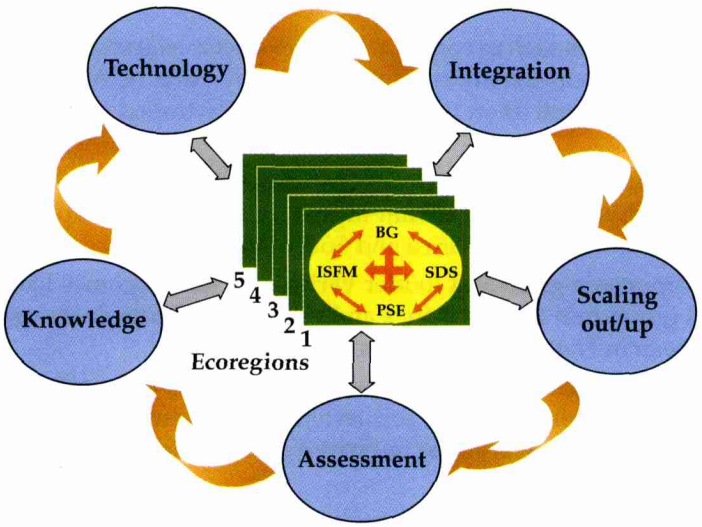

Figure 1. CP-BNF implementation strategy with multidisciplinary approach in target ecoregions, including integrated soil fertility management (ISFM), legume-*Rhizobium* biodiversity and genomics (BG), seed delivery systems (SDS) and policy and socioeconomic issues (PSE).

- West and Central Africa
- East and Southern Africa
- North Africa and West Asia
- South Asia
- Latin America.

Starting with a detailed analysis of past successes at implementing N_2 fixation technologies (Component 1), targeted research on specific topics relating to these case studies through basic research (Component 2) will lead to advancement of understanding of these systems. Gaining fundamental knowledge within all disciplines will then underpin the development of promising technologies (Component 3) and provide the tools and developments required for integration (Component 4). The latter will lead to the design of new systems, and system components to be implemented through dissemination at various levels (Component 5). The whole process is seen as an iterative mechanism with each component intimately linked and providing continuous feedback to the others.

Component 1. Analyzing Success Stories

Significant impacts on livelihoods and on sustainability of farming systems have been achieved in the last decade using a variety of N_2-fixing legumes. Examples of notable successes include:

- grain legumes such as common bean (*Phaseolus vulgaris*) as a key source of food protein for the rural poor in many parts of Africa and Latin America, as well as an important commodity traded for cash in many regions;
- multipurpose legumes such as pigeonpea that provide grain for food or sale, fuelwood and significant inputs for soil fertility improvement in eastern and southern Africa and South Asia;
- multipurpose grain and fodder varieties of food legumes like cowpea and groundnut in West Africa and the indeterminate, leafy varieties of soybean in Nigeria and southern Africa;
- legume forages and fodder trees such as *Leucaena* and *Calliandra*, which provide protein-rich feed, soil fertility and fuelwood, that have been widely adopted as feed for improved dairy cattle in many countries in the tropics; and
- soil fertility improvement through improved fallows of legume trees in eastern Zambia, western Kenya and Asia.

CP-BNF will document and conduct detailed interdisciplinary analyses to determine the reasons for the success of these legume-based technologies

and their relative impact. Some of the factors that will be considered are the role of local and improved legume germplasm, dependence on indigenous rhizobia or inoculation of strains, impacts of past research, major biophysical constraints and means of alleviation, agroecological targeting of technology, mode of dissemination of the technology (including seed systems), socioeconomic and health impacts on different income groups, institutional environment, economic and policy frameworks, and long-term impacts on sustainability of farming systems. The research emphasis during this component of the CP-BNF will be on understanding the underlying components of success to allow rapid replication of the impact in new areas.

CP-BNF will not focus solely on mainstream grain legumes, but strive to utilize new tools and available knowledge in selection and improvement of forage and tree legumes, including neglected and underutilized legume species. Locally important successes of herbaceous legumes such as *Arachis pintoi* and *Mucuna puriens* in Central America and of minor grain legumes such as moth bean (*Vigna aconitifolia*), Bambara groundnut (*Vigna subterranea*) and cluster bean (*Psophocarpus tetragonolobus*) will also be assessed because they often occupy important niches in marginal and dry environments. We will then be in a position to identify potential candidates for other regions. Attention will also be given to economically important N_2-fixing trees that form symbioses with *Frankia*, notably species of *Casuarina* and *Alnus*.

Exploitation of legume N_2 fixation through CP-BNF will be linked to whole system production and sustainability goals. Systems based on efficient use of fixed N have the potential to be environmentally friendly and to contribute to important ecosystem services such as the provision of the abundant amounts of N required to sequester C in soil organic matter.

Component 2. Gaining Knowledge

CP-BNF is also committed to enhancing our fundamental understanding of N_2 fixation across disciplines, including socioeconomic and policy analysis, integrated soil fertility management and molecular physiology. In addition, gaining fundamental knowledge within all disciplines will underpin the development of promising technologies in Component 3. This work will be strongly driven by and coordinated with research in Component 1 and in the target areas.

Farming system-scale analyses of the N cycle, including detailed assessments of amounts of N_2 fixation and availability of that N to subsequent crops will employ stable isotope (^{15}N) methods, coupled with long-term studies on soil organic matter pools (using ^{13}C where appropriate). Simulation modeling at various scales will be used as an integrative tool for linking the various processes and understanding impacts on the long-term sustainability of technologies and effects on productivity of other crops within the system.

These analyses will be conducted within a structured evaluation recognizing local variability in inherent soil fertility.

Strong links to socioeconomic analyses of the production and social systems, and their local agroecological, social and institutional context will assist in understanding the appropriateness of technologies for farmers differing in natural and social resource endowment.

Ecophysiological tools and approaches will provide a conceptual framework for characterizing the plant-rhizobia-environment status using selected legume species and genotypes in each reference production area. The mechanisms and underlying genes that influence the host-rhizobia-environment interaction are being rapidly elucidated in model legume species. This will lead to critical new tools for manipulating tolerance for abiotic stress (and resistance to biotic stresses).

Studies using polyphasic taxonomy will allow the diversity of microsymbionts for legumes in the reference zones to be characterized. Study of interactions with other functional groups of rhizospheric microorganisms, both beneficial (e.g., mycorrhiza, *Trichoderma*) or detrimental (pathogens), will be used to explore whether other functions of rhizobia (like phosphate solubilization or siderophores) have beneficial effects on the N_2 fixation and growth of the host legume. This will form part of detailed research to understand the non-N benefits of breaking monocultures of cereals with legume rotations, such as suppression of *Striga* and other weeds, reduction of pest and disease incidence and stimulation of populations of beneficial organisms.

Component 3. Capturing Synergies from New Technologies

Rapid development of new technologies for the future will be based on the analysis of past successes and the knowledge and understanding based on process research. The guarantee of success of these technologies will be founded in a strongly farmer-driven and orientated selection of production goals. Whole system assessment of the bio-pedoclimatic factors that limit SNF and its contribution to the N cycle (and balances of other nutrients) and the soil organic matter will allow understanding of effects on long-term system sustainability. Identification and direction of breeding and selection priorities will be an important contribution of this farmer and environmental analysis.

Vast investments are being made in basic research of model legume species in the USA, Europe, Japan and Australia. A fundamental cornerstone of this Challenge Program is the CGIAR's pivotal role in rapidly translating this fast-growing knowledge for direct benefit and application to the lesser-studied legumes. The successful application of these technologies will be entirely focused on farmer-driven priorities.

Existing technologies from modern genomics and bioinformatics will play a major role in the rapid development of legume germplasm best adapted for N_2 fixation in the face of major abiotic stresses (drought, phosphorus deficiency, soil acidity, alkalinity) and biotic challenges (pests, diseases, parasitic weeds). The speed, precision and cost effectiveness of germplasm characterization and enhancement will be substantially increased through the implementation of existing physiological knowledge coupled with the application of molecular marker technologies.

Current initiatives in model legume genomics will play an essential role in fueling the progress of CP-BNF. Exploitation of the synteny between genomes will allow marker-assisted selection for biotic and abiotic traits to be rapidly developed and applied in ways that were previously impossible. Ongoing research on the creation of a consensus genetic map for the major tropical legumes (*Phaseolus, Glycine, Vigna*) through the PHASEOMICS project will have direct impact on tropical beans and cowpea, but must also be extended to the many green manure and forage species from the tribe Phaseoleae (e.g., *Mucuna, Centrosema, Calopogonium*). Similar initiatives have been established around the model species *Medicago truncatula* for the Mediterranean species of agricultural importance (*Medicago, Trifolium, Pisum,*) and these must be extended to those related crop species of importance to the poor, such as chickpea and lentil.

The power of synteny is exemplified by the recent identification of two genes in *Lotus* that are essential for nodulation and N_2 fixation. Researchers were able to rapidly identify the parallel genes in pea. An international consortium is needed to provide overall integration and translation of these initiatives. This consortium must also ensure that the genomics of lesser-studied crops is sufficiently developed to enable the type of immediate uptake of advances in the model systems as already seen in pea, alfalfa and soybean. Construction of genetic maps for these major legume groups will focus new attention on legumes of major importance in the agriculture of developing countries (with the exception of *Arachis*).

Our philosophy regarding the bacterial microsymbionts will be to manage the diverse populations of tropical soils to ensure effective nodulation of a wide range of legumes. However, some selection of efficient, adapted strains will be done where inoculation is required. Other rhizosphere functions of rhizobia and their interaction with beneficial soil organisms such as mycorrhiza are further opportunities to explore.

Component 4. Integration

Past research on legume N_2 fixation has largely been driven by a commodity-based plot- or field-scale approach, despite the increasing realization that natural resource management has to be tackled at the system scale. For

example, various projects have explored the potential of grain legumes, herbaceous green manures or multi-purpose trees for improving soil fertility, but virtually no studies exist where the potential of all of these approaches is compared within a single study or target area. Farmer-led evaluations of suites of promising N_2-fixing legume-based technologies will lead to rapid understanding of local adaptation and potential from the variety of available legumes.

A major CP-BNF thrust will be to break down commodity-related barriers in the CGIAR institutes, other international organizations and NARS to allow a focus on the wide role of legume N_2 fixation within the farming systems in stimulating productivity and contributing to sustainability. Opportunities for introduction and expansion of legume-based technologies will be based within a whole systems approach to ensure an optimal contribution to productive and sustainable agriculture. This approach is fully endorsed in the mid-term plan of the African Association of Biological Nitrogen Fixation (AABNF 2001).

By focusing activities within the CP-BNF on selected target regions, the broad range of skills required for interdisciplinary research will be mobilized from international and national research institutes. Robust technologies and economic conditions are required for successful integration and widespread uptake. Well-adapted legume genotypes with multiple disease and pest resistance are a key resource for all successful legume-based systems. Economic institutions and policies, ensuring effective input supply and marketing chains must be in place. Thus, excellent research on a broad suite of topics will underpin application of technologies based on legume N_2 fixation.

Full understanding of the benefits of N_2 fixation requires detailed assessment of the multiple roles of legumes in grain for food or sale for cash, fodder, reduction in labor demands due to weed control and soil fertility benefits. These benefits are often competing, such that an essential stage in evaluation of new technologies is analysis of various types of trade-offs associated with adopting new technologies for farmers. One example of this is weighing demands for labor against production and soil fertility benefits. Another is comparing the short-term benefits for food and the inputs of N available for following crops with long-term effects on soil fertility. Because the usefulness of different technologies differs between farmers, it is necessary to evaluate a wide range of options.

A focus of CP-BNF on target areas in each ecoregion will not restrict activities to those areas because successful technologies piloted in the target areas can readily be adapted for new conditions. The use of simulation modeling together with strong agroecological and socioeconomic characterization (linked to GIS databases) will be used to explore appropriate extrapolation domains and assist rapid selection of suites of technologies for testing in new areas. The approach will essentially be one of prototyping

new N_2-fixing legume-based whole farming systems with farmers within the target areas.

Component 5. Scaling up and Scaling out

Dissemination of the strategies and technologies developed under CP-BNF into farmers' fields is central to the program. A participatory extension program for BNF and complementary soil fertility management technologies will be implemented on the basis of:

- the analysis of the opportunities for enhanced application of N_2-fixing legume-based technologies (Component 1),
- new BNF and legume technologies developed under the CP (Components 2 and 3); and
- extensive consultations with farmers and other stakeholders (Component 5).

The program will be implemented by existing extension agencies. Because several BNF technologies can only effectively be applied in combination with other soil fertility management techniques (e.g., P application), CP-BNF will propose methods for improving agricultural production and maintenance of soil fertility based on BNF as well as complementary techniques. Through regular consultation and links with farmers and researchers, the dissemination program will continuously adjust to new developments to ensure that it reflects the demands of farmers.

Specifically, the project will support the implementation of participatory training programs by local extension providers, both government agencies and NGOs. Partnerships with international NGOs will be sought to ensure widespread impact and dissemination of BNF technologies. Local extension providers will be trained in the technical aspects of soil fertility management through enhanced application of BNF and complementary techniques, as well as in the participatory training aspects. These training programs will be disseminated from at least 10 key sites in different agroecological zones.

This component will also focus on capacity building of existing research and extension networks. A strategic alliance will be forged with the FAO for implementation of participatory approaches. Annual regional workshops in each target area will review progress and ensure coordination of all activities. Four symposia will be held within the timeframe of the CP (every 2-3 years).

To prepare the next generation of researchers with an appropriate range of skills, the CP will support the training of a substantial number of PhD students and postdoctoral fellows, both at NARS and at the CGIAR centers. A detailed program for capacity building on legumes and BNF has been proposed by IFS to be part of the CP-BNF.

All research and capacity-building activities will be undertaken in continuous communication with farmers and through extensive in-field testing of new technologies and wider evaluation of available technologies. Regular training courses in legume N_2 fixation will be held under the auspices of the AABNF and RELAR in English, French and Spanish to satisfy the need for non-degree based training.

OUTPUTS, ACTIVITIES AND EXPECTED IMPACTS

The five-component strategy of the CP-BNF will be implemented by the interactions of the various disciplines involved in BNF research at target areas in each ecoregion. The target areas, based on key success stories, will guide the selection of activities for each discipline. Progress in different components will be strategically linked so that all activities/outputs will be driven and evaluated in a participatory farming systems approach.

OUTPUTS:

A. Integrated Soil Fertility Management

- Legume technologies evaluated, tested and transferred for greater use of N_2-fixing plants in cropping systems, crop rotations and intercropping
- Participatory methods for dissemination of BNF technologies and better adoption of integrated natural resource management options
- Improved options for soil fertility management developed and disseminated
- Integrated options for crop-livestock systems productivity developed
- Agroforestry/legumes systems for marginal environments developed

B. Legume-Rhizobia Biodiversity and Genomics

- New paradigms in legume breeding (using novel combinations of conventional and biotechnology-assisted approaches)
- Enhanced knowledge and ability to manipulate BNF efficiency and legume productivity
- A pipeline of legume germplasm with increasing N_2-fixing efficiency and adapted to stress plus high and stable food and fodder productivity

C. Seed and Inoculum Delivery Systems

- Seed and inoculum supply constraints identified according to agroecology and national/sub-regional boundaries

- A breeder and foundation legume seed and inoculum supply system established on a regional basis
- Legume end-user needs identified that can be addressed through seed supply interventions to differentiate product markets based on grades and standards
- Information on leguminous tree propagation documented and disseminated
- Rhizobial inoculum quality control and regulatory systems developed
- New institutional arrangements developed around public-private partnerships that bring about sustainable innovation in seed and inoculum supply systems

D. Policy, Markets and Socioeconomic Issues

- Understanding of the limiting and enabling factors for increased legume production
- Policy options and institutional strategies for improving the impact of legumes developed
- Increased demand, marketing and utilization systems for grain legumes
- Stakeholder participation in innovation systems institutionalized
- Decision support systems for policy analysis

The main users of CP-BNF outputs will be resource-poor farmers, worldwide crop-livestock producers and consumers, policymakers, and NARS. The potential for increasing BNF through enhanced legume utilization will make a substantial contribution to the sustainable increase in food production and improved soil fertility. The outputs of this programme will contribute significantly to the development of a holistic approach for sustainable crop-livestock production. This will in turn reduce yield gaps and soil fertility gaps, without draining soil nutrients while minimizing inputs from external sources. Furthermore, this will help resource-poor farmers to overcome the socioeconomic limitation regarding access to chemical fertilizer, by providing alternative or complementary sources of nitrogen. Increased productivity will contribute to higher and more sustainable on-farm incomes, reduced poverty, better health conditions and livelihoods.

PARTNERSHIPS, CONSULTATION AND STAKEHOLDER PARTICIPATION

During Formulation of the Pre-proposal

The CP-BNF will build on past research achievements and impacts, including the outcomes of the Eco-Regional Alliance (ERA) on legume improvement

(CIAT, ICARDA, ICRISAT and IITA) on legume crops. This initiative includes over 65 CGIAR scientists working on various aspects of legume production and utilization, in collaboration with a number of NGOs, extension providers, and NARS. The main research topics addressed are genetic resources and breeding, agronomy and microbiology, plant protection, quality and post-harvest processing and socioeconomics.

This pre-proposal also builds on the experiences gained during an international workshop on BNF convened by FAO in March 2001. One of the outcomes of this workshop was that there is an urgent need for enhanced application of BNF and that in the short, medium and longer term important opportunities exist to improve rural livelihoods through BNF and complementary soil management. Finally, the pre-proposal draws upon the experience of those USA universities and CGIAR centers forming the Global Legume Genomics Initiative created through a workshop at USDA in September 2001.

Based on these initiatives and consultations, an interactive website has been established to facilitate communication and exchange of information between the various partners and stakeholders (http://www.icrisat.org/bnf/Biological.htm). Two working sessions were organized at the first International Conference on Legume Genomics and Genetics (Minneapolis, June 2002) with around 100 participants showing considerable interest in this initiative (http://www.agro.agri.umn.edu/iclgg).

In consolidating the experience and vision of these groups for the preparation of the BNF pre-proposal, an international stakeholder consultation workshop was hosted by INRA-ENSAM and Agropolis in Montpellier, France (June 2002). This meeting was attended by some 35 researchers and development experts each representing key disciplines and institutions. After an extensive review of the status of legume and BNF research in the various ecoregions, position papers from the four ERA partners were presented, summarizing the past achievements and current challenges of research on legumes. Working group sessions were then organized to prepare the pre-proposal of the CP-BNF. The CP mission, goals, objectives and contribution activities were thus successfully developed with joint participation of all participants through working groups and plenary discussions.

Stakeholder consultation has continued with presentation and discussion of the CP-BNF initiative in various regional and international meetings, including the CORAF/WECARD General Assembly (July 2002 at Yamoussoukro, Côte d'Ivoire), the 1st International meeting on microbial phosphate solubilization (July 2002 at Salamanca, Spain), and the 5th European Nitrogen Fixation Conference (September 2002 at Norwich, UK). It will also be discussed during the 10th Conference of African Association of BNF (AABNF) in Accra, Ghana (October 2002).

The consultation will continue through a permanent stakeholder dialogue process. The papers presented at the Montpellier workshop will be edited and published to serve as background documents for the next steps of CP-BNF development.

During Full Proposal Preparation

On acceptance of the pre-proposal, the consultative process leading to the final proposal will be initiated. A stakeholder workshop will be organized, that will include representatives from the various CGIAR centers, FAO, development experts, relevant academic researchers, and representatives of the NGO community and private sector. During this workshop, the process for the final development of the proposal will be agreed and working groups established. These working groups, comprising leading scientists and representatives of the users of BNF technologies, will prepare a background paper addressing the current status of BNF research, as well as a detailed list of activities to be carried out during the CP-BNF.

During Implementation

Stakeholder consultation is at the core of CP-BNF. Stakeholders, particularly farmers, will have an important say in selecting technologies for further development. In addition, the status of developments, including an integration of research outcomes and an examination of the comparative advantage of different techniques (Component 2), will be assessed biennially. Farmers' representatives will provide important inputs in these assessments by relating their experiences with BNF application. Moreover, all technologies will be tested in farmers' fields, allowing farmers to provide direct feedback on their costs and benefits (Component 3). Finally, the dissemination process (Component 5) will be based on participatory extension techniques such as farmers' field schools, where farmers select appropriate techniques.

A major means of implementation will be through regional networks directly related to the GFAR: CORAF ASARECA, and SACCAR for FARA, implemented through the AABNF and TSBF-AfNet; FABAMED for the Mediterranean Basin and Nile Valley; PROFRIJOL, PROCARIBE and TROPILECHE for the Caribbean basin and Central America; PROFRIZA for South America, and CLAN and Biofert (Japan) for the Asia-Pacific region.

RELATIONSHIP WITH OTHER CHALLENGE PROGRAMS

CP-BNF could have an important win-win relationship with a number of other candidate challenge programs, including those on Genetic Resources, Biofortification, Climate Change, Desertification, and Livelihoods in sub-

Saharan Africa. Because CP BNF focuses on the improvement of legume productivity and the reduction of the soil fertility gap through N_2 fixation and integrated soil fertility management, it will have the specificity and potential to provide significant scientific input concerning the role of legumes in the cropping systems under investigation by other CPs.

- Biological N_2 fixation and legume intensification are especially critical in areas with poor soil fertility, such as sub-Saharan Africa. Because the legume crops, shrubs and trees in these environments are targeted for investigation, a strong and synergetic interaction is envisioned with the candidate CPs on Desertification and Livelihoods in Sub-Saharan Africa.
- CP-BNF will assist in the development of a series of initiatives such as the System-wide Livestock Program coordinated by ILRI and the NUANCES (Nutrient Use in Animal and Crop systems - Efficiency and Scales) initiative led by Wageningen University.
- The genomics components of the Genetic Resources CP, which deals mainly with cereals, will complement the CP-BNF focus on legumes.
- Legume intensification will increase the human intake of protein, iron and zinc, leading to improved health of the rural poor, particularly women and children. Linkage with the Biofortification CP will bring synergies for better understanding and use of these legume qualitative traits.
- The role of legumes and potential benefits of BNF in cropping systems under climate change scenarios could open new and exciting research areas on C/N plant metabolic relationships, and carbon sequestration, and lead to major scientific breakthroughs.

The details of the relationships between CPs will be further investigated and resolved during full proposal development.

STRUCTURE, GOVERNANCE AND MANAGEMENT

The proposed challenge program will operate with a lean management structure, taking full advantage of the BNF networks, sub-regional fora and international biotech initiatives to administer its operations. This will ensure that management overheads and transaction costs are reduced to an absolute minimum, while maximizing feedback and orientation by stakeholders. A secretariat consisting of a coordinator and a secretary will provide the central focal point for the CP-BNF and will use regular (quarterly) newsletters to ensure information flow amongst partners.

A Scientific Advisory Council (SAC) will be constituted by scientists from key centers of expertise in each of the disciplines involved in the CP. The

SAC will be chaired by an eminent international scientist and will act in an advisory and resource capacity to support the CP steering committee.

The CP Steering Committee (SC) will be the highest policy body for the challenge program. It will be composed of representatives designated by partner institutions and charged with the responsibility of overall coordination and streamlining (Fig. 2). The SC will be co-chaired by the chair of SAC and the Director General of ICRISAT. The SC will define and amend the program mission and policy statements and report to the CGIAR and other stakeholder organizations. The program coordinator of the CP will be act as secretary to the SC, and assume primary responsibility for programme management, coordination, fundraising, and advocacy. The final constitution of the SC will be discussed with the CP partners and stakeholders during the full proposal development.

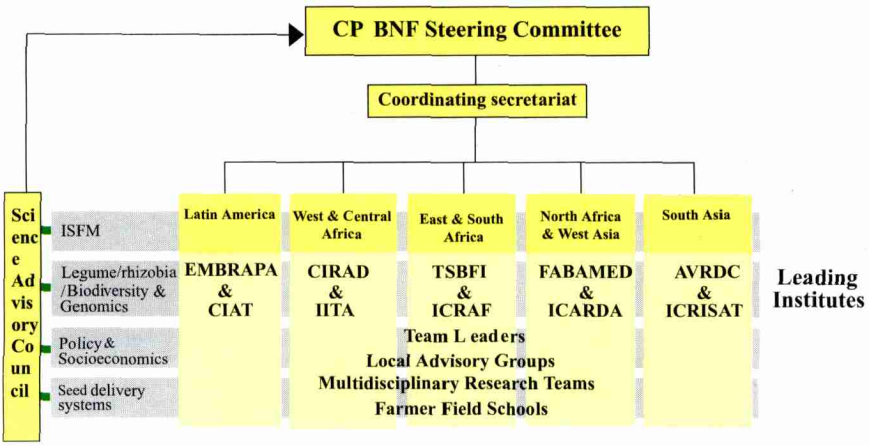

Figure 2. Proposed management mechanisms for the CP-BNF.

The program will be implemented through multi-disciplinary multi-sector regional teams in each of the five target regions, formed from a wide range of expertise and partners (CGIAR, regional and subregional fora, NARS, ARIs, NGOs and private sector institutions). Each ecoregional sub-program will be under the co-leadership of one CGIAR center and one non-CGIAR research institutions. The regional multidisciplinary teams will link to and be drawn from existing networks in each region that will act as conduits for broader stakeholder consultation and dissemination through regular scientific workshops.

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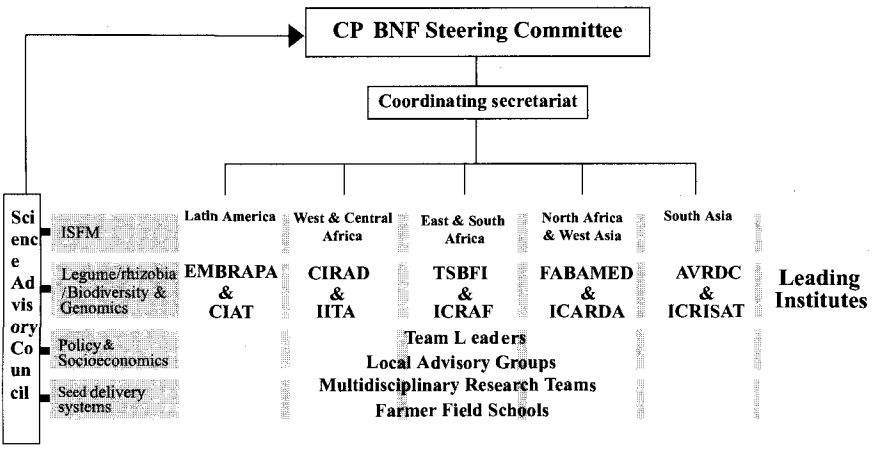

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